

How to lose with nothing to lose

NATO is making heavy weather of battlefield weapons in Europe. It would find it easier to modernize its forces if it would negotiate as well, and that way would also keep its friends.

MR Dick Cheney, the new US Secretary of Defense, will not win many friends in Europe with his statement at the weekend that if the West were to embark on negotiations on short-range nuclear weapons, "there's a very real danger" that the end result would be a "de-nuclearized" Europe. "What would be so wrong with that?" is how many people will respond. Especially if there is also a cast-iron deal on the numbers and character of the conventional forces deployed in Europe by the Warsaw Pact and the North Atlantic Treaty Organization (NATO). In short, Cheney's remark may serve only to strengthen the growing body of opinion in West Germany whose clamour sent Hans-Dietrich Genscher, the Foreign Minister, and Gerhard Stoltenberg, the Defence Minister, scurrying from Bonn to Westminster to put the case for negotiations.

NATO, and the United States and Britain in particular, are in danger of talking themselves into a false position on this issue, which is neither new nor obscure. NATO's European strategy, which goes back to the early 1960s, is explicit, even correct. Supposing (or at least believing) that the Warsaw Pact's forces are the stronger on the ground, Western strategy has relied on battlefield nuclear weapons to beat back an overwhelming conventional attack. But now there are serious negotiations under way at Vienna on the mutual regulation of conventional forces, launched propitiously by the Soviet Union's unilateral decision last December to reduce its armed forces by 500,000 men and women and to withdraw 15,000 tanks from the European region. NATO is well within its rights to say (as it does) that those reductions will not make a stable Europe, and to ask for more to match whatever reductions are offered by the West. But if there is a chance of reaching an acceptable balance on conventional forces, and if short-range nuclear weapons are meant to make up for the West's perceived deficiencies in that area, it must make sense to talk about both at the same time.

So why is NATO so hesitant? One consideration is its wish to replace some of its battlefield weapons with modernized Lance missiles (with a range of 500 kilometres) and, no doubt, to remove many others from Europe altogether. The West German government has been a reluctant partner in that process because many of those who elected it to office would prefer negotiations to modernization, and might elect another government next

spring if they do not get their way. Sympathetic to Chancellor Helmut Kohl's difficulties, some other NATO members have been saying that the decision about Lance could be postponed until after the elections. That explains why the NATO communique last week spoke only of bringing battlefield nuclear weapons "up to date", without saying how or when. West Germany agreed. The significance of the form of words has since been fully explained by NATO governments, Cheney's among them: it amounts to a postponement of a decision about Lance until after the West German elections. Yet West Germans read newspapers as avidly as anybody. If Genscher, one of the minority Free Democrats in the West German Cabinet, has concluded that this form of words, and its intention, has provided the opposition parties with a blunderbuss, that merely shows that he is an experienced politician. No wonder he wants a new understanding.

NATO's fear of negotiations on battlefield weapons is harder to understand. Cheney spoke as if negotiations could have only one end-point: abolition. The logic is elusive. Who believes that the conventional arms negotiations will ban tanks from Central Europe? Why cannot the West appreciate that negotiations on battlefield nuclear weapons could, instead, be an opportunity to convince the Warsaw Pact (and even West German electors) that Lance missiles, being less in danger of conventional capture than, say, nuclear artillery, might be the best embodiment of Western weapons?

Cheney also seemed to argue at the weekend that negotiations are to be avoided because the Soviet Union has always wanted a "de-nuclearized Europe". It is true that Mr Mikhail Gorbachev has sometimes echoed his predecessors in saying that. But what Gorbachev has recently been asking is that there should be negotiations. His argument is cogent (see *Nature* 338, 527; 13 April 1989), and deserves a constructive answer. As time passes, Cheney will find it easier to say "yes" than "no".

The unspoken part of the dialogue is that which affects the nuclear weapons of European states themselves, Britain and France. So far, neither's nuclear planning has been much affected by arms control negotiations. The unratified SALT II treaty, which constrained allowable numbers of Soviet and US strategic missiles, implicitly took account of these peripheral forces, but without direct commitment by their operators: ironically, the



moral force of the treaty is such that the major powers abide by it, but Britain and France can build what weapons they choose. The British Prime Minister, Mrs Margaret Thatcher, has said she will consider negotiating about British weapons when the major powers have a strategic agreement, which will not be tomorrow. Her French counterparts have said nothing. Yet each of them will also have to say "yes" at some stage. □

Oppressed press

Lacking constitutional protection, British newspapers are under pressure to reform — or be regulated by law.

AMERICANS concerned with press freedom are constantly surprised to find the British government banning books and suppressing news. No doubt that is why, last week, Mr Dana Bullen, the former *Washington Star* journalist who heads the World Press Freedom Committee, told an International Press Institute conference (called to discuss growing press censorship in Britain), that much of the new British Official Secrets Act is "unconstitutional".

Such parochialism, in the days of *Spycatcher*, is no longer excusable. Britain does not have a First Amendment any more than it has a Grand Canyon or a President. The US Congress may pass no law restricting freedom of the press. The British Parliament can. One private member's bill, which would have given a statutory right of reply to those who claim to be victims of inaccurate reporting, was rejected last week. Another — protecting privacy — is expected also to fail. But there is to be an independent inquiry into the conduct of British newspapers. The press, said Mr Timothy Renton of the Home Office, announcing the inquiry, has a year or two "to clean up their act". If not, Parliament will do what Congress cannot.

In last week's debate, much rage was vented against the newspapers, particularly the tabloids. But Renton, among other top Tories, expressed a deep reluctance to introduce restrictions of a kind not seen since the time of Cromwell. The Thatcher government, nonetheless, shows no inhibition at all in trying to censor the much-more-regulated medium of broadcasting. As things are, shocking as it is to many outside Britain, legally elected representatives of the legal Irish nationalist organization, Sinn Féin, including one elected to the Westminster Parliament, are banned from British radio and television. (At least, their voices are: pictures may be shown, with others speaking their words.) But the anger at the supposed damage done by the media could be extended to curbs on the press.

Those who deplore this prospect should keep in mind three factors that do not exist in the United States. One is active terrorism, ready to strike at any time. The Prime Minister was nearly killed by the Provisional IRA at Brighton in 1984 (and five of her colleagues perished). Earlier, one of her closest friends had been killed by a bomb within the precincts of Parliament itself. The second

factor is that there is a large number (eleven) of national newspapers, engaged in a bitter circulation war with each other; many will do anything to gain an advantage over their nearest rival. The third is the royal family. These unfortunate people, unlike pop stars or television personalities or politicians, are ordinary in every way except birth. Yet the smallest detail of their private lives can sell millions of papers. Privacy laws, such as those in the United States, protect people not in the public eye, on the assumption that celebrity is somehow voluntary. It will be hard in Britain to draw up comparable laws because of this royal anomaly.

Hope that legislation may be avoided lies in the intellect and personality of the new chairman of the Press Council, Mr Louis Blom-Cooper, QC. An ardent admirer of the First Amendment and a civil libertarian lawyer of international repute, Blom-Cooper has, for the past few months, led the British newspaper and magazine industry's own voluntary self-appointed regulatory body. He has sworn to make the council more responsive to public alarm. Last week he made an excellent start, by deciding that the council will conduct its own immediate inquiry into the photographic coverage by the tabloid newspapers of the Hillsborough disaster, when close on 100 people were crushed to death in a football stadium. The front-page full-colour close-ups of the faces of dying teenagers distressed many people.

The serious national newspapers — the broadsheets — consider themselves unjustly blamed for the sins of the sleazy tabloids, all lumped together as the unloved 'press'. But the so-called 'qualities' are not blameless. They seem arrogant. They do not carry correction columns of routine errors. They do not have ombudsmen to whom the public may take its grievances. And they do not editorially scold the tabloids when these — as Mr Rupert Murdoch's do — refuse to publicize the rebukes the Press Council gives them.

The British government is not blameless either. It has done nothing to prevent the concentration of newspaper ownership, which only intensifies the tabloids' circulation wars. Murdoch, who already owns *The Times*, the *Sunday Times*, the *Sun* and the *News of the World*, was allowed also to buy *Today* without the sale being referred to the Monopolies and Mergers Commission. And the Prime Minister has bestowed a knighthood on the editor of the *Sun*, apparently forgiving him for its pornographic daily page three because of his paper's staunch Toryism.

The best outcome of the rocky year ahead would be for the Press Council and the independent inquiry to persuade the national newspapers that they must make themselves visibly more responsive to public complaints. That might stave off worse. Bullen last week supposed the virtues of the US system to be so plain that it must have been copied elsewhere. But it has not been. The British Parliament should not make laws telling British newspapers what and what not to print, but is in a mood to do so, and there is no constitution to prevent it. British newspapers, beware. □

Hopes for nuclear fusion continue to turn cool

■ Press conferences continue

■ Verification mostly halting

Washington

If the withdrawal by Stanley Pons and Martin Fleischmann of the cold fusion paper submitted to *Nature* bolstered rising scepticism, enough happened this week to keep hopes alive. Claims of successful fusion arrived from California, India and Brazil, and Pons himself hinted at new experimental evidence. But at the press conferences at which the announcements were made, hard data have been lacking and conclusions unclear; still no verdict can be reached.

One positive note is the publication in this issue of the more modest claim by Steven Jones and his colleagues, at Brigham Young University, who give evidence for a small but significant increase in neutron flux when current is passed through a palladium cathode in a suitable electrolytic cell. Jones and his group believe that fusion is occurring, but at a level much below what Pons and Fleischmann need to explain their energy production rate.

That claim is now at the centre of the debate. Neither in the published paper nor in the version submitted to *Nature* is there enough detail for readers to make their own estimations of the workings of the electrolytic cells. That and some other relevant issues are dealt with elsewhere (see pages 701, 705, 710 and 711).

At a press conference in Salt Lake City on 17 April, Stanley Pons announced a qualitatively new piece of supporting evidence: mass spectroscopy of the gases evolving from a working fusion cell revealed the presence of ^4He in quantities consistent with the reported energy production, if all deuterium-deuteron fusions produce ^4He rather than tritium and a proton or ^3He and a neutron.

Cheves Walling, a colleague of Pons, says that gas from the cell was sampled and analysed, and a production rate of 10^{12} atoms per second was deduced after the total gas flow rate had been figured in. This rate of helium production is much greater than in the 1926 'cold fusion' experiments (page 692), in which about 10^{10} atoms were detected after several hours.

Walling and a colleague, John Simons, have submitted to the *Journal of Physical Chemistry* a theoretical paper suggesting how the high density of electrons in the palladium lattice could allow the high-energy photon expected, with a ^4He nucleus, to be converted into lattice vibrations and thus heat. Although Walling

describes the theory as 'qualitative', he says that enhancements by many orders of magnitude of this normally rare fusion mode could be achieved.

At the 17 April press conference, Pons addressed an issue which has caused disquiet since it was brought up by Harold Furth, director of the Princeton University Plasma Physics Laboratory, at the recent meeting of the American Chemical society in Dallas, Texas (*Nature* 338, 605; 1989) — the lack of a direct comparison between an electrolytic cell containing heavy water and one containing ordinary water. Pons tantalized his audience by indicating that preliminary results from just such a comparison suggested an "unexpected" production of heat in the ordinary cell. There are no numbers.

A similar comparison between 'light' and 'heavy' electrolytic cells was described at Stanford University on 18 April by Robert Huggins, of the Materials Science Department. He said that when two cells, identical except that one used H_2O and the other D_2O , were run side by side with the same voltage applied, the heavy-water cell consistently ran the hotter by as much as 15 per cent. Huggins says that differences in the specific heat of heavy and light water, or in the diffusion rates of hydrogen and deuterium into the palladium electrode, are not large enough to explain the results.

Other groups are attempting similar comparisons, but none has yet reported success; Huggins says that there are "a couple of features" in his set-up that others may have missed, but these will not be revealed until a special session of the meeting of the Materials Research Society, scheduled for the evening of 26 April in San Diego.

In India, two groups claim to have achieved cold fusion in electrolytic cells similar to those used at the University of Utah. A third group, at the Bhabha Atomic Research Centre (BARC) in Bombay, is setting up a large-scale electrolysis reactor expected to produce results towards the end of May.

C.K. Mathews and colleagues at the Indira Gandhi Atomic Research Centre (IGARC) at Kalpakkam near Madras have reported neutron production from electrolysis of heavy water using a platinum anode and a titanium cathode. The team used an electrolyte of nickel and palladium chlorides at 0.2 per cent concentration rather than the lithium salt used by Pons and Fleischmann. "We observed a

30 per cent increase in neutron flux over the background level, suggesting that fusion was taking place", said IGARC director Dr C.V. Sundaram. No measurements were made of energy output. Sundaram said the results were not always reproducible because the experimental parameters had not been optimized.

In an almost identical set-up at the Tata Institute of Fundamental Research in Bombay, researchers claimed to have observed energy amplification, but they did not look for neutrons. Professor K.S.V. Santhanam and his colleagues in the chemical physics group said that passage of 0.25 watts of electrical power through the cell produced one watt of thermal output at the titanium cathode, whose temperature rose to 80°C in a sustained reaction. Heavy water mixed with sodium chloride was used as the electrolyte and platinum as anode in this experiment.

BARC's director, Dr P.K. Iyengar, said his group is setting up a much larger version of the Utah experiment with sophisticated instrumentation for measuring heat as well as fusion products. Results are expected in a month. But Iyengar, a well-known reactor physicist, is sceptical.

India is one of the few countries producing heavy water in commercial quantities and may therefore have an advantage if cold fusion is real. BARC is also interested in cold fusion as an inexpensive neutron source, which could be used, instead of fast-breeder reactors, to transmute India's 360,000-tonne reserve of thorium into uranium-233 fuel. But BARC's spirit has been dampened by reports that, whatever happens inside palladium, it does not produce a large neutron flux.

In Brazil, two groups have announced, by the now-traditional press conference, that they have obtained fusion reactions while trying to replicate the Utah experiments. The first team to report, from the Physics Institute of the University of São Paulo, said on 19 April that it had detected neutrons at twice the background level. Spero Penha Morato says that only a deuterium-deuteron reaction could account for this excess of neutrons, but the team was not equipped to measure heat production.

The second announcement, on 29 April, from the Institute of Space Research (INPE) in São Jose dos Campos in São Paulo state, says that a group headed by Gerson Otto Ludwig and known for work on fusion by magnetic confinement has reported two separate neutron bursts during a 100-hour experimental run. The first burst, after ten hours, had an intensity of ten times background; the second, after 35 hours, four times. At another press conference on 21 April, the same group announced it had detected ^3He , thus proving that fusion reactions had occurred. □

1920s discovery, retraction

Munich

WHILE researchers around the world spend sleepless nights in the laboratory trying to reproduce evidence of cold fusion, reports from the United States of a 1926 'mystery paper' on cold fusion in the German-language literature have sent more reflective physicists scurrying to their libraries.

Paul Allison and Klaus Lockner, of Los Alamos National Laboratory, began the chase by sending out an informal translation of a paper from the respected German researchers Fritz Paneth and Kurt Peters, in the 1920s at the Chemical Institute of the University of Berlin, reporting the creation of helium from hydrogen using a palladium catalyst. But the results were retracted eight months later, after new sources of error were identified.

The episode exhibits striking parallels to the current furor over cold fusion. The theory of the day could not explain the results of Paneth and Peters, but there was good reason at least to attempt the transmutation of hydrogen into helium. And the news caused great excitement, as indicated by the response in *Nature* (see excerpts reprinted on page 706).

Little was understood of thermonuclear fusion in 1926, although Paneth and Peters do mention in their introduction to their paper the hypothesis that helium is produced from hydrogen in stars. But neutrons were discovered only in 1932, and physicist Hans Bethe demonstrated in 1933 that fusion was the likely source of stellar energy.

Paneth and Peters published their results first in the journal *Berichte der Deutschen Chemischen Gesellschaft* (59, 2039; 1926). The results were then reprinted in *Die Naturwissenschaften* (14, 956; 1926). This paper and its 1927 retraction (*Berichte der Deutschen Chemischen Gesellschaft* 60, 808; 1927), reprinted in *Die Naturwissenschaften* (16, 379; 1927) are models of clarity. A letter of retraction was also sent to *Nature* (119, 706; 1927).

The technique for the spectroscopic detection of amounts of helium as small as 10^{-8} cubic centimetres was by far the most difficult part of the experiment, and had taken 'several years' to develop. The first stage, in which helium was 'created' from hydrogen, was more a shot in the dark, although palladium was recognized as a catalyst.

At first, Paneth and Peters passed about 1 litre of hydrogen gas through a red-hot palladium capillary to "create" helium "spontaneously". But when they noticed that spectral lines of helium could be seen even when the capillary was at room temperature, they simplified their apparatus. They exposed hydrogen to a number of palladium preparations — a 'black', a

sponge or palladinized asbestos — for various periods of time. After twelve hours, enough helium was formed to show four or five spectral lines.

Paneth and Peters tried hard to account for possible errors in their method. For example, helium might have been trapped inside the palladium. They convinced themselves that this could not be so by repeatedly exposing their palladinized asbestos catalyst to hydrogen and oxygen. Only in the presence of hydrogen was helium released. Therefore, they concluded, the helium must have derived from hydrogen and not from an experimental artefact.

Unlike Pons and Fleischmann, Paneth and Peters did not observe the release of large amounts of heat from their apparatus. They write that they would have expected only a fraction of a calorie of heat to be produced by the creation of 10^{-8} cubic centimetres of helium. Paneth and Peters conclude that the energy must be released in the form of radiation, but add that they had not detected it.

In April 1927, came the retraction. Paneth *et al.* had tested their results at Cornell University and in Berlin and drew the conclusion that they had "underestimated" two sources of error.

The first clue emerged during experiments designed to check whether helium could have diffused from the atmosphere through the glass walls of the apparatus. While performing numerous control studies, Paneth *et al.* found that glass heated in a hydrogen atmosphere yielded up absorbed helium, in amounts of about 10^{-9} cubic centimetres, whereas glass heated in a vacuum yielded none. Helium detections at this level, they concluded, were to be discounted.

The second blow was the realization that the palladinized asbestos catalyst that had given the best results was, like glass, a considerable source of helium, which it released readily in the presence of hydrogen, but not in that of oxygen. In an almost self-mocking tone, Paneth *et al.* write that they must strike from their results all the trials with a palladinized asbestos catalyst, in which helium was 'created' in amounts up to 10^{-7} cubic centimetres, and upon which they had earlier placed "particular value".

The story also has an addendum which parallels modern activities. In February 1927, John Tandberg of the Electrolux Research Laboratory filed for a Swedish patent on a device which produced "helium and useful energy". This invention was an electrolytic cell, using ordinary water, based on the work of Paneth and Peters but with a "significant increase in efficiency". The patent was never granted.

Steven Dickman

Complicity alleged against physicist

Munich

A Max-Planck Society physicist has been implicated in illegal exports to Pakistan of sensitive nuclear technology. Testimony given before a parliamentary committee in Bonn on 20 April revealed that the physicist, a prolific inventor who received a number of patents for himself and his employer, the Max-Planck Institute for Plasma Physics (IPP) in Garching, may be prosecuted for his involvement in the exports. He was dismissed from his post on 17 April when the institute's directors learned the extent of his involvement.

It had been known since December that Pakistan, South Africa and India had received nuclear technology from the West German companies NTG (Neue Technologien GmbH) and PTB (Physikalisch-Technische Beratung) without the necessary export licences having been issued by the West German government.

The physicist was also mentioned in the news as a consultant for NTG. But IPP defended him at the time, saying that his consulting work did not go beyond installing a tritium-removal apparatus using the so-called TROC process (Tritium Removal with Organic Compounds). TROC is used to remove tritium from glove boxes and other work areas. It cannot be used for the production or holding of the large amounts of tritium that can intensify the effect of a nuclear bomb. The physicist had developed TROC and IPP holds the patent on it.

But last week it emerged that the physicist also helped NTG install a "tritium handling system" for holding large amounts of tritium free from contamination. The use of this system for bomb production cannot be ruled out, though by itself it cannot produce tritium. The physicist did not inform IPP of his activities nor did he say that he had formed his own consulting firm.

The technology exported to South Africa and India could have been used only for peaceful purposes, said Hanau prosecutor Albert Farwick, who is handling the case against NTG, which is located in Gelnhausen, in the Land of Hesse. Pakistan denies that it broke West German export law.

The decision whether to prosecute the physicist will be reached by the autumn, said Farwick, who said that the physicist was "in no way the head" of the export operation. The case has generated some sympathy for the physicist, who is looked upon by some of his IPP colleagues as a "tragic case".

The physicist, who is estimated to have earned about DM50,000 for his work for NTG, is appealing against his dismissal by IPP. His case is expected to be heard in two weeks.

Steven Dickman

Press pleads for more basic science

Washington

THE new US presidential science adviser D. Allan Bromley is "walking into a situation unparalleled in opportunity", according to US National Academy of Sciences president Frank Press, himself a presidential science adviser during the Carter presidency.

Bromley, Press said, will have a chance to accomplish real advances in the way the US science enterprise is financed and managed. Press, speaking in advance of his annual address to the members of the academy, said that the Bush administration's concern for maintaining US strengths in science and technology, coupled with the increased political power of the science advisory post, could make Bromley's tenure the most impressive in recent history.

Press's hints that the next few years may be a watershed for US science were borne out in perhaps the most sweeping of his yearly speeches to the members of the science academy. In marked contrast with last year's address (*Nature* 333, 1; 1988) in which he stressed the importance of setting priorities for science within the confines of a budget, Press called for doubling US spending on basic science over the next five years to \$20,000 million.

Press says that could be done by raising the growth rate of the basic science budget from 6 to 14 per cent per year, at a total cost to the United States of \$10,000 million. Such a hefty rise should be possible, says Press, if big expenditures are delayed until after the federal budget deficit is reduced.

To remove the barriers blocking the industrial benefits of such a large investment in science and technology, Press proposes the creation of a cabinet-level National Economic Security Council with the same powers over the high-technology economy that the National Security Council has over defence. His view is that the presidential science adviser would play a key role in directing the activities of the council.

Press also advocated a "national facilities act" to provide the infrastructure necessary for major science developments. The legislation would pay for refurbishing laboratories, instruments and equipment. Press estimates the equivalent of 50 new buildings could be had for \$1,250 million. Press also advocates the creation of 3,000 graduate fellowships in science and engineering, at a cost of \$3,000 million.

But Press's grand proposals may not be taken up as enthusiastically as he would wish by Congress and the Bush administration. President Reagan's stated goal to double the US National Science Foundation budget over five years has not yet been attained. But Press has the patience to wait: if his suggestions are not taken up this year, he plans to pursue them again in 1990.

Carol Ezzell

Presidential adviser named

Boston & Washington

AFTER more than two months of delay and increasingly vocal protest from the scientific community, US President George Bush last week finally announced his choice of Yale University professor D. Allan

IMAGE
UNAVAILABLE
FOR COPYRIGHT
REASONS

D. Allan Bromley, new science adviser.

Bromley for the post of presidential science adviser.

Bromley's appointment continues a tradition of favouring physicists for the post of adviser to the president and director of the Office of Science and Technology Policy. Bromley, who will be 63 next week, is Henry Ford II Professor of Physics and director of the A.W. Wright Nuclear Structure Laboratory at Yale University. He was born in Canada and has a high reputation in heavy-ion physics where he has been active in the development of accelerators, detectors and computer-based data collection and analysis systems.

Bush's failure to keep his promise to select a science adviser early in his administration had already prompted congressional protest by the time of the Bromley appointment. On 6 April, at a hearing of the Senate Labor and Human Resources Committee called by Senator Edward Kennedy (Democrat, Massachusetts), a series of witnesses, including Frank Press, president of the National Academy of Sciences, and two former science advisers, Donald Hornig and Guy Stever, expressed their disappointment that a science adviser had still not been appointed and that few of the 500 key federal administrative posts in science had been filled.

But Bromley says that Bush's campaign promise to upgrade the status of the science adviser is now to be fulfilled. Following "a discussion with the president" on 21 April, Bromley confirmed that "the new post will be the assistant to the president for science and technology. The science adviser will have direct access to the president and will be on the same level as the national security adviser [Brent Scowcroft].... This means that I will participate in meetings of the National Security Council, and I will be present at meetings that had not involved past science advisers." Bromley says he will not try to bring his own priorities to the

job but rather "sharpen options for the president" and "be part of the president's team". "Any science adviser who is perceived as a spokesperson for the scientific community is doomed to failure", he says. "The science adviser must be trusted in all detail by the president, must participate in policy debate, and be present in high-level decisions that have a relevance to science and technological matters." Numerous problems with a strong scientific component face the president, including the need for decisions on the Strategic Defense Initiative, the space station, the Superconducting Super Collider, the project to map the human genome, the space plane, and international action to counter global atmospheric change.

Even more difficult, because the choices are not clear, will be the setting of national policy to restore the United States' technological superiority and industrial competitiveness in the face of the growing strength of Japan and Western Europe. "This issue leads quickly to concern for education and training", says Bromley. "The questions of science and technology today are integrated across a whole spectrum of governmental affairs. It is very different from Eisenhower's day when the position was created. Now, agencies across the board are very sophisticated in their use of science and technology." So many agencies are now involved in science that some experts believe that a science adviser cannot hope to have much impact and that a new science organization is required. In testimony before Senator Kennedy, Donald Hornig, former adviser to President Lyndon Johnson, pointed out that the Office of Science and Technology Policy has never had much influence over the National Institutes of Health, with their \$7,000 million budget, nor over the Department of Defense, where two-thirds of the federal research and development budget (\$42,000 million) is spent.

The present science adviser, William Graham, claims to have spent much time nurturing inter-agency communication. But Graham's soft-spoken style gave him a reputation for near-invisibility. Colleagues of Bromley describe a man who is likely to act very differently, speaking of him as "emphatic", with new students initially "in awe of him".

Bromley directed former President Ronald Reagan's science and technology initiatives with India and Brazil and stresses the importance of international science. A key question, he says, is whether the United States should embark on huge projects "alone as a nation, or whether we should involve other countries".

Seth Shulman & Alun Anderson

Gupta affirms authenticity

New Delhi

PROFESSOR V.J. Gupta, of the Panjab University at Chandigarh, has angrily denied charges by Dr John Talent in last week's *Nature* (338, 613; 1989) that he invented fictitious fossils and had rewritten the geological history of the Himalayas through dubious publications. Gupta claimed that Talent's outburst was motivated by "a malicious intent to take revenge for personal rivalry and professional jealousy over the past 20 years".

Gupta did not specify Talent's motives for revenge, but implied that his interest in the Himalayas went beyond geology, and that the attack on him had something to do with information he was unable to provide to Talent. Gupta said his fossil collections came from Himalayan sites that were either militarily sensitive or close to international borders and thus not open to foreigners.

According to Gupta, Talent and his associates "wanted some information [about localities] which we cannot provide [for security reasons]". He said that Talent visited India 20 times "always entering from Pakistan", but says that he did not publish any paper on India. "I wonder which agency would finance a scientist for so many travels that result in no scientific output?" he asked.

On the authenticity of his fossils, Gupta says they were dug out by a team, not by him alone. To get them identified, expert opinions were sought from palaeontologists abroad. In some cases, opinions were contradictory, but "the diverse opinions were kept in view while working out the stratigraphic set-up of the area, taking account of the geological succession exposed in the field from where the fossils were collected". He said Talent was biased in his opinion about the ages of stratigraphic units.

Replying to the charge that his details of the localities at which his fossils were collected are distorted, Gupta said he had had to depend on old and inaccurate maps whose use is restricted by the government for security reasons. Gupta said his 25 years of work has documented the existence of Silurian-Devonian succession in the Himalayas and that "the truth about it will be proved by the progeny during the forthcoming years".

Predictably, the Talent-Gupta controversy has evoked a spate of comments from Indian scientists and hurried meetings at the Indian Geological Survey. Gupta's colleagues at the Centre for Advanced Study in Geology, including its director, Dr A.K. Prasad, described Talent's allegations as "a conspiracy to denigrate a top Indian scientist".

But the Society for Scientific Values (SSV) plans to launch its own investiga-

tion of the allegations. Describing them as "serious and undermining of Indian science", the society's president, Dr A.S. Paintal, said it will appoint an expert team to make an impartial evaluation of Gupta's work.

Contrary to Talent's implied remarks,

CFCs

Japan supports ban by the year 2000

Tokyo

JAPAN will support a total ban on the use and production of chlorofluorocarbons (CFCs) by the year 2000, Japan's Environment Agency announced last week.

Japan's decision, to be formally announced next month in Helsinki at a meeting of signatories of the Montreal Protocol, follows similar pledges to end the use of CFCs made last month by the United States, the European Community and Canada (see *Nature* 338, 101; 1989). Japan's support will be important for the success of a total ban as it is one of the largest producers of CFCs, accounting for about 10 per cent of world production.

The Environment Agency has been keen to support revision of the Montreal Protocol and the announcement represents a victory over the powerful Ministry of International Trade and Industry (MITI). MITI is concerned that Japan's semiconductor industry may be seriously affected by a ban. Japan is the world's largest consumer of freon 113, the CFC used to clean semiconductors, and produces approximately 130,000 tonnes annually. Alternatives to

India does have a mechanism to check fraudulent research, said Paintal, who is also chief of the Indian Council of Medical Research. With a membership of over 120 reputable scientists, SSV was set up two years ago with the aim of improving the quality of science in India. So far, it has been concerned largely with allegations of misconduct in biomedical research.

K.S. Jayaraman

freon 113 are still in the early stages of development.

The machinations that led to the Environment Agency victory are unknown, but the words of an eminent foreign scientist and Japan's emperor may have helped.

Earlier this month, Frank Sherwood Rowland of the University of California, who was in Tokyo to receive the Japan Prize for his pioneering research on the link between CFCs and ozone depletion, repeatedly called for a worldwide ban. His appeals received wide media coverage.

At the presentation ceremony for the prize, the Emperor in a congratulatory speech noted that when CFCs were first synthesized for practical application, no one suspected that they would have such "dire consequences" he said that this should be a reminder that "science and technology need to be constantly monitored and appraised from newer and wider perspectives".

It is unusual for a Japanese emperor to make a public statement on such a politically sensitive subject. Following such a speech it would have been odd if Japan had not supported a ban.

David Swinbanks

ANIMAL RIGHTS

Anti-terrorism bill introduced in Senate

Tucson

CONGRESSIONAL legislation that would make it a federal crime to steal or harm research animals or to break into and damage research facilities was introduced on 7 April in the US Senate.

Alabama Democrat Howell T. Heflin proposed the Animal Research Facility Protection Act of 1989 so that federal law enforcement agencies can become involved against what he said could be an international conspiracy.

During the next month, the Senate Committee on Agriculture, Nutrition and Forestry will review the bill, which will be an amendment to the Animal Welfare Act. Passage into law could take more than a year. The legislation also makes unauthorized entry into facilities and possession, control or duplication of records, data, material, equipment or animals a possible federal offence. Each violation could lead to a one-year prison sentence and a \$5,000 fine. The district court or magistrate could force the guilty party to

pay for damages and for reasonable cost of repeating ruined experiments. The research institution could also sue for damages.

The legislation also calls for the Secretary of Agriculture and the attorney general to assess the extent and effects of "domestic and international terrorism" on animal research, production and processing facilities. That survey would include places where animals are on exhibit or used in food production or as pets. A report would be due within a year.

Heflin said he had been working on the bill for several months at the insistence of the University of Alabama in Birmingham, but that a recent incident at the University of Arizona in Tucson (see *Nature* 338, 534; 1989) "acted as a catalyst to bring about a more rapid introduction than we had planned". In that incident, a group calling itself the Animal Liberation Front set fires in two university buildings, vandalized laboratories in two other buildings and took about 1,200 small animals.

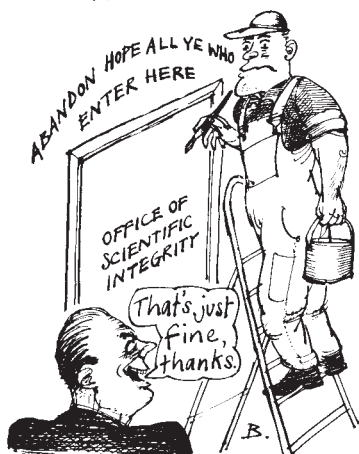
Elizabeth Pennisi

SCIENTIFIC MISCONDUCT

New office gets going

Washington

THE National Institutes of Health (NIH) Office of Scientific Integrity is changing from an administrative requirement into a reality. It has been given space on the crowded NIH campus, an acting director has been appointed and staff are being



assembled. But all this is happening despite threats from Congress that the office should be moved out of NIH to another agency that can be more objective about misconduct in biomedical research.

NIH and other federal agencies have been labouring hard over the past several months to find better ways of coping with scientific misconduct. Although few in the research community believe that the problem is widespread or growing, congressional and public interest has been fostered by several recent well-reported cases of alleged misconduct.

Earlier this year, the Public Health Service decided to create two new offices to deal with misconduct (see *Nature* 338, 588; 1989). The Office of Scientific Integrity in the office of the director of NIH will oversee — and conduct when necessary — misconduct investigations, recommend punishments and promote high standards of research conduct. The Office of Scientific Integrity Review will be in the office of the Assistant Secretary for Health of the Department of Health and Human Services, and will oversee the NIH office.

Brian Kimes, associate director for extramural programmes in the division of cancer biology and diagnosis at the National Cancer Institute, will become full-time acting director of the NIH office on 1 June, and will run the office on a part-time basis until then. Initially there will be a staff of eight, including two secretaries. Kimes expects that it will take about a year to clear the backlog of some 40 misconduct cases that are pending.

Although some cases will always take time to resolve, Kimes says a priority will be to speed up processing of the more routine cases. He also believes that as institutions receiving grants become more

adept at dealing with issues of misconduct — partly through experience and partly from guidance provided by NIH — fewer cases will require attention from NIH.

Kimes has had previous experience in misconduct, having participated in the investigation of Mark Spector, a graduate student at Cornell University. He says cutting-edge research that is somehow adulterated is easy to spot because many laboratories will quickly attempt to replicate it. Far harder to detect and punish is research that lies outside the main stream, published in journals that do not provide careful refereeing of submitted work.

Despite action by NIH, there still exists the possibility that legislation will be enacted to move the office out of NIH and into the office of the assistant secretary for health. Leslie Russell, a staff member of the powerful House of Representatives Energy and Commerce Committee, told an audience earlier this month, at a colloquium on science and technology of the American Association for the Advancement of Science, that such legislation will be introduced soon. Russell said NIH may face a conflict of interest in looking into misconduct research.

New regulations that spell out the responsibilities of federally funded institutions for investigating misconduct will be published in the near future. They are at present awaiting approval at the White House Office of Management and Budget.

Joseph Palca

EUROPEAN COLLABORATION

25 years of ESA

Paris

THE European Space Agency (ESA), whose headquarters are in Paris, last week added another birthday celebration to those of the Eiffel Tower and the French Revolution. It is now 25 years since European countries officially pooled resources to form the European Space Research Organisation (ESRO) and the European Launcher Development Organisation (ELDO). These merged in 1974 to become ESA (see page 718).

Helmut Kohl, the West German Chancellor, who was in Paris for a Franco-German summit, applauded ESA's success and invited the agency to promote its role in Earth observation, both to further efforts in pollution control and also to monitor progress in disarmament — a role its constitution probably excludes. French President François Mitterrand saw ESA as also a triumph for France, the biggest partner and principal driving force behind the commercial launch sector, Arianespace, as well as the transportation elements (Hermes and Ariane 5) of Europe's Columbus contribution to the US Freedom space station.

Nobel laureate Carlo Rubbia meanwhile reminded the distinguished gathering of scientists and diplomats from ESA's 13 member states that CERN (the European Laboratory for Particle Physics), ESA's 'elder brother' in Europe's family of large organizations, was established by some of the same scientists as was ESRO.

Peter Coles

CHARITIES

UK cancer fund finds new ways of making money

London

THE Imperial Cancer Research Fund (ICRF), one of Britain's largest medical research charities, has substantially increased direct expenditure on research in the 1980s, by increasing its income and eating into its investment reserves. Next year, those reserves are expected to reach £14 million, the minimum desirable, and Sir Walter Bodmer, ICRF's scientific director, now says any new plans for expansion will have to wait for three to four years until income exceeds expenditure. In the annual report, published last week, the treasurer reports a 27 per cent increase in direct expenditure on research last year, which left income almost £7 million less than expenditure, a deficit met from reserves.

Increased income has come from a new initiative — charity shops. Since 1983, ICRF has set up 200 shops, which now bring in £4 million a year. By the late 1990s, the fund hopes to have 600 shops bringing in £16 million and to double income received from legacies to £72 million.

Major new projects under way include new laboratories at the Institute of Molecular Medicine in Oxford, for research into solid tumours; a new laboratory at the University of Cambridge for research into a vaccine for cervical cancer, by the ICRF's tumour virus group; and a new unit at the Institute of Psychiatry at Maudsley Hospital, London, to investigate smoking behaviour as well as examining the effects of passive smoking on children.

Expansion of the ICRF's main laboratories at Lincoln's Inn Fields, in central London, is also under way. A new building for administration and computing facilities will free space for 50 laboratory bays. Conversion into laboratories is due to begin in 1990 and will cost £3.3 million. Uncertainty about the funds that will be available could mean that completion will be slower than planned, a maximum of four years instead of three. But Bodmer says this development has top priority in the, and stresses that no existing commitments will be cut to meet costs of this expansion.

Christine McGourty

Democracy finally wins

Moscow

ON 20 April, the Soviet Academy of Sciences finally elected its quota of deputies to the country's new parliament, bringing to an end the four-month election campaign to the Congress of People's Deputies that will form the Soviet parliament.

Members of the Soviet Academy had wanted to be represented by socially orientated legislators, people with radical reform programmes in their pockets, who would firmly and boldly struggle against the administrative and command system, rather than talking about *perestroika*.

The election procedure in this public organization has evoked much interest both in the Soviet Union and abroad. In March, the academy's election conference annulled the decision of the extended plenum of the academy's leadership, held last January, to nominate 23 candidates for 20 vacancies and to ignore a long list of names advanced by numerous academic institutes. Then, only eight candidates won the necessary number of votes (at least one more than the number entitled to vote), while the other 12 seats remained vacant. This triggered the legal requirement for the follow-up elections.

The academy's leadership learned the lesson. They took into account proposals advanced by academic institutes, invited the press and the pressure group called "For Democratic Elections in the Soviet Academy". Voting by a show of hands, they nominated 25 candidates for 12 seats. (On the last day before the vote, the well-known Soviet economist, Academician

Stanislav Shatalin, withdrew from the elections for health reasons.)

The election conference was attended by 1,101 voters, including Soviet academicians, non-voting members of the academy and 433 representatives of academic institutes. The voting was preceded by heated debates: all candidates presented their political, social and legal programmes and answered scores of tough questions about the most pressing issues of *perestroika*.

The support of grass-roots organizations proved decisive. Those elected were, among academicians, Andrei Sakharov, Roald Sagdeev, Vitaly Ginzburg and Georgy Arbatov and, among non-voting members of the academy, Sergei Averintsev, Pavel Bunich and Nikolai Petrakov. Other successful candidates were doctors of economics Nikolai Shmelev and Gennadi Lisichkin, both well-known authors of strongly worded articles; Dr Alexander Yakovlev, a lawyer; Yuri Kariakin, head research worker of the Institute of the International Labour Movement; and Dr Viacheslav Ivanov, a philologist.

On balance, the Soviet Academy of Sciences has passed its examination in democracy with flying colours. Undoubtedly, its deputies to the Soviet Parliament as well as scientists elected from other public organizations, territorial and national-territorial districts, will represent a powerful group working to speed up the radical restructuring of domestic and foreign policies.

Yuri Kanin

NUCLEAR REACTORS

Superphénix go-ahead

Paris

Superphénix, the French fast-breeder reactor, has been given the go-ahead to begin generating electricity again after a two-year interruption while a structural fault was investigated. Earlier this year, the reactor was restarted without its leaky fuel-rod transit chamber, despite protests from environmental groups in neighbouring Switzerland who want the plant to be closed down.

Following trials without producing electricity, the French nuclear safety authorities are satisfied that the generator can resume full-power output (1,200 MW) within the next two months. But environmental groups are outraged that no independent inquiry has been carried out and say that to restart the generator without the transit chamber will leave the plant with no safe site to allow fuel rods to cool in the event of an accident. A new transit system for fuel rods is being installed and will be ready for the autumn.

Peter Coles

AIDS

Controversial visa ban

Washington

The US policy of denying entry visas to foreigners infected with the human immunodeficiency virus, the virus which causes AIDS, may become a contentious issue before next year's international AIDS conference, scheduled to take place in San Francisco, California. Several researchers are expected to introduce a resolution at this year's conference in Montreal, Canada to move the 1990 conference to a country that does not screen travellers for HIV infection.

The United States passed a law two years ago prohibiting immigrants and aliens infected with HIV from routinely entering the country. Several US congressmen have said that the intent of the law was to turn away HIV-infected immigrants seeking permanent residence, but the law recently was invoked to detain a Dutch city official with AIDS who was travelling to the United States for a health conference.

Carol Ezzell

Test flight of IRBM abandoned after postponements

New Delhi

INDIA'S eagerly awaited test flight of its first intermediate-range ballistic missile (IRBM) did not take place on 20 April as scheduled, disappointing dignitaries including the defence minister and the three service chiefs who had come to witness the launch from Chandipur in coastal Orissa state. The launch time was postponed three times and late in the afternoon the Defence Research Development Organisation (DRDO), which developed the missile, announced that the test had been put off indefinitely. No reasons were given, although unofficial reports said the mission was aborted because of technical problems.

The two-stage, 100-tonne missile named Agni is said to have a range of 2,500 km and to be able to deliver a warhead weighing more than a tonne. Except for the inertial navigation system, for which France provided help, the missile is claimed to be fully indigenous. The first stage is solid-fuelled for quick acceleration and the second is propelled by a storable liquid of very high specific impulse.

Although not officially stated, the main aim of the launch was to evaluate the performance of the heat shield — an important piece of hardware in a weapon delivery system — during its re-entry through the atmosphere.

The missile, in fact, had been named after this important mission objective (*agni* means fire in Sanskrit). Although DRDO had developed the heat shield some time ago, its testing had to wait until Agni was ready.

After Trishul and Prithvi, Agni is the third missile to roll out of the DRDO laboratories in Hyderabad. Trishul is a short-range (12-km) surface-to-surface missile.

The postponement of the IRBM launch after much publicity is a setback to DRDO which had hoped to score over Pakistan's recent success with its HATF-2 missile, which brings Bombay and Delhi within range. Prithvi, DRDO's long-range surface-to-surface missile, has a range of just 250 km. It has yet to enter the production phase.

DRDO would have launched Agni secretly had there been no population near the test range. As it turned out, opposition by some 12,000 villagers to mass evacuation from their homes in the safety zone brought the event under the glare of national and international publicity. Meanwhile, DRDO has not given any indication of when it will fire Agni next.

K.S. Jayaraman

Hitachi opens new labs

Tokyo

HITACHI Ltd of Japan last week announced the opening of four research laboratories in the United States and Europe that will form close ties with nearby universities. Hitachi's move is the latest in a series of steps by Japan's industrial giants to form links with universities in the West, and it has raised fears that Japan is trying to tap the brains of Western academic institutions. But university researchers involved in the collaboration see the joint research as mutually beneficial.

Hitachi's new laboratories, which will open within the next few months, will be in the Cavendish Laboratory of the University of Cambridge in the United Kingdom, the O'Reilly Institute of Trinity College at the University of Dublin in Ireland, and within Hitachi America Ltd at San Francisco and Detroit in the United States. The arrangement with the University of Cambridge is particularly novel.

The Cavendish Laboratory, one of the world's leading research institutes with a string of Nobel prizewinners to its name, has been desperately seeking funds for a new building for its microelectronic researchers who are currently housed on a separate site at the Science Park in Cambridge. The lease on their building in the park runs out in September and cannot be renewed, according to Dr H. Ahmed, head of the microelectronics laboratory.

Unable to get support from the government, the university has had to dig into its trust funds and turn to Hitachi and another Japanese company for help. Hitachi has donated £250,000, or nearly a third of the £800,000 needed for construction of the building, and Japan's Toshiba Corporation has also given £50,000, says John Deakin, secretary of the Cavendish Laboratory. Construction is expected to be completed in December.

Toshiba's donation is not unusual. Many companies, for example Britain's Rolls-Royce, have contributed to research at the University of Cambridge. What is unusual is that, in return for its large donation, Hitachi will gain control of about a quarter of the new building with its own private entrance.

Hitachi will set up joint research projects with University of Cambridge researchers. The first couple of Hitachi researchers will arrive in May and will collaborate with Ahmed and one of his students on the computer simulation of future microelectronic devices. When the new building opens in December, Hitachi will increase its laboratory staff to about ten, including several British researchers, and the company will pump in annual research funds of about ¥200–250 million (about £1 million), says Hitachi vice president Hiroshi Watanabe. The research

centres in Ireland and the United States will be of similar size. And within about ten years, Watanabe hopes to expand the total number of researchers in Europe and the United States to about 200, dispersed among small laboratories of 10–20 researchers, with most of the staff recruited locally.

Hitachi is just one of several Japanese companies that have recently established research centres linked to universities in the West. Other examples include NEC Corporation, which will open a laboratory in Princeton, New Jersey next month that will carry out joint research on information technology, such as automatic translation machines, with Princeton University. Kobe Steel Ltd opened a polymer composites research laboratory at the University of Surrey in the United Kingdom last October. And Hitachi Chemical will use two-thirds of the space in a new biotechnology laboratory of the University of California in exchange for a \$12 million donation.

Many Japanese companies have for

several years been making donations to leading US universities, for example through the endowment of university chairs. But the move to establish joint research centres is a new phenomenon and there are fears in the West that it is an attempt by Japanese companies to pick the brains of the Western world.

Ahmed, however, sees the collaboration in quite a different light. Japan leads the world in much of the research and development of microelectronics. As well as Hitachi gaining access to the University of Cambridge, Ahmed's student will spend six months in Hitachi's Central Research Laboratory in the outskirts of Tokyo which is equipped with one of the finest clean rooms in the world.

Hitachi's local subsidiary will retain patent rights to all joint research carried out with the University of Cambridge. But the Cavendish Laboratory will get "about 50 per cent" of patent royalties under its agreement with Hitachi, says Deakin. And both Deakin and Ahmed are confident that the university will gain both financially and academically from its collaboration with the Japanese company.

David Swinbanks

MARINE SCIENCE

Australia to seize the seas?

Sydney

A NEW report on Australia's marine industries recommends a radical reorganization of the nation's marine science and technology capabilities and calls for Australia to claim its Exclusive Economic Zone, an area bigger than its land mass. But the federal Department of Science appears to be in no hurry to submit to cabinet the report's recommendations, which would cost A\$11.7 million to put into effect. The full report will be made public shortly.

Increasing interest by foreign governments in Australia's fishing zones, the growth in visitors to the Great Barrier Reef and the potential of off-shore oil and gas prompted the government last year to convene a committee chaired by Professor Ken McKinnon, vice-chancellor of Wollongong University.

Marine industries are at present worth more than A\$16,000 million annually, of which A\$4,500 million is gross export income. The federal government spends A\$70 million a year on marine research and development but there has been no growth for four years.

In line with the government's view that industry should help pay for the development of technology, the report recommends upgrading of areas such as aquaculture, marine biotechnology and seabed mineral assessment. "The picture which emerges is that our efforts in the marine field are unbalanced. A large proportion

is concentrated on marine biology and too little in the physical sciences and technology. There are vast amounts of money to be made in marine technology fields, the incentive for industry is untapped profit", McKinnon said.

The report says that previously unharvested species of fish could be exploited in Australia's Exclusive Economic Zone and calls for a personal levy on tourists visiting the Great Barrier Reef "to help maintain the reef in its original state". The committee proposed the formation of a statutory Australian Marine Industries and Sciences Council (AMISC) with responsibility for policy, planning and funding. Eighteen different government departments have an interest in marine sciences, according to the report, so there is likely to be disagreement as to which should take responsibility.

Joe Baker, director of the Australian Institute of Marine Science (AIMS), welcomed the recommendations. "Overall the report brings together data that has been, until now, scattered. We recognize that there must be the incentive for industry to invest in the marine environment and we recognize that, ultimately, AIMS, together with the oceanography and fishing divisions of the Commonwealth Scientific and Industrial Research Organisation and some components of the Bureau of Meteorology and the Antarctic Division will have to be incorporated into a larger structure."

Tania Ewing

Theory versus practice

SIR—As a mere data-generator, I am no doubt ill-equipped to follow the deep theoretical meditations of Virginia A. Huszagh and Juan P. Infante ("The hypothetical way of progress"; *Nature* 338, 109; 1989), far less to criticize them. I am, apparently, caught up in a labyrinth of authoritarian ideology, stale paradigms and pseudo-hypotheses; nor is there anyone who could show me the way out of this maze, as all the creative minds have been driven off, and my fellow-prisoners are mediocre imitators who are unable even to make simple distinctions between speculation and theory. As a wretched product of such a dismal environment, shall I be forgiven for advancing the non-scientific and sociologically based view that this was one of the most inane pieces to be published in *Nature* in many months?

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SIR—Huszagh and Infante make several valid points in their plea for more encouragement for theoretical papers in biology. However, as the editor of a journal (*BioSystems*) which receives (and encourages) theoretical contributions, I take exception to the generalization that journals and reviewers tend to dismiss theoretical work out of hand. As Huszagh and Infante themselves acknowledge, there is much *pseudo*-theorizing, and the overall rejection rate in this area is probably justifiably high. More might be done to stimulate interactions between theoreticians and experimentalists by broadening our overspecialized science curricula, rather than by awarding grants.

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SIR—It is with great interest and an immense sense of relief that we read the Commentary by Huszagh and Infante. The authors echo our own fears and experiences as PhD students. A PhD thesis seems meant solely to be a repository of unwieldy data — and data that conforms with the literature at that.

Things are not very different at seminars. Research data are presented and a particular explanation is offered where two or more possibilities might coexist and they are all dismissed on the grounds that "such examples are not found in the literature". Does the past trend of results govern the future course of science?

It is of course man's ability to think that changes the course of science, not the mere compilation and arrangement of data. Albert Einstein said: "... it appeared that it was possible to get certain knowledge of the objects of experience by means of pure thinking ... Nevertheless, for anyone who experiences it for the first time, it is marvellous enough that man is capable at all to reach such degree of certainty and purity in pure thinking as the Greeks showed for the first time to be possible in geometry".

RADHA SRIRAM

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New kidneys

SIR—The leading article (*Nature* 337, 393; 1989) about replacement kidneys reaches a conclusion with which few would disagree, namely that better international arrangements are needed for collecting, matching and delivering kidneys to where people need them. In reaching this conclusion, the writer cites two reasons why a commerce in replacement organs should not be unthinkingly banned.

It would be imprudent to ban any practice unthinkingly, but if it is accepted (perhaps naively) that the role of the state is to ensure the well-being of its people, the first reason, as exemplified by the case of a parent who sells an organ in order to provide proper care for a handicapped child, becomes less compelling. An indigent parent is naturally willing enough to do all in his or her power to help a handicapped child, but society should not require that such a parent be exploited by the organ trade. The second reason given is that a law aiming to ban a commerce in replacement organs is unlikely to be effective. Similar objections were probably raised to laws against the slave trade. Admittedly, it is quite possible to draft an ineffective law, but much more frequently it is the enforcement of a law that is ineffective because enforcement depends to a large extent upon political will.

We are then left to consider the morality of a commerce in replacement organs. Commerce implies profit and an indigent who sells an organ is unlikely to profit from the sale. I do not know of any study of the longevity of organ donors, but it is possible that donation may adversely affect the life expectancy of a donor. If loss of life expectancy occurs during the earning period of a vendor's life, the profit from an organ sale that accrues in the long term to a seller is apt to be minimal.

There is reason to believe that a signifi-

cant part of the profit from the sale of a replacement organ ends up not with the person who is the source of that organ but with the entrepreneur who arranges the deal. While the donation of an organ is an act greatly to be admired, reason requires that an exploitive commerce in replacement organs be speedily proscribed by internationally accepted and enforced legislation.

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Apoptosis or apoptomeatic

SIR—The use of the word 'apoptosis' to describe the active death of a cell is not new. The term is first attributable to a poet, Dioscoriades, who used it in the first century to describe the seasonal falling of flowers¹. The Greek medical authors Hippocrates and Galen use the word to denote hair loss in balding gentlemen, or the weakening of bones with age. All these events could be caused by an active, programmed cellular response. Greater artistic licence was taken in the second century, with Esais, another Greek poet, using 'apoptosis' to refer to 'falling by the wayside' or 'backsliding' in a theological sense². This is presumably the origin of the definition located by Kleine³. Modern English dictionaries do not list the word, but contemporary medical ones do; they dissect it into 'apo' for 'head' and 'ptosis' 'to fall', with particular reference to balding. This may be an artificial division, but the exact origins of the term are unclear.

The 'apoptotic' T lymphocyte is something of a linguistic menagerie. 'Lympho' is derived from 'limpa', a Latin word for 'clear water', the 'p' being corrupted through Greek influence to 'ph' (just as the nypae became the nymphae). 'Cyte' on the other hand is a true Greek word meaning 'hollow vessel'. Clearly the process of activated cell death deserves accurate description: an alternative Greek word is 'apoptomeatic', this being an 'unlucky chance' or 'loss' as used by the authors Polybius⁴ and Athaneus⁵. However, as it is not mere fortune but instead a specific antibody that initiates cell death, as described by Smith *et al.*⁶, 'apoptosis' remains the more reasonable alternative. Perhaps 'apoptosis' should join the growing list of scientific terms for review in standard English dictionaries.

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1. Dioscoriades *Prooem* 1, 8.

2. Esais *Parologia Graeca* 34, 4.

3. Kleine, B. *Nature* 337, 402 (1989).

4. Polybius *Historicus* 11, 26.

5. Athaneus *Mathematicus*.

6. Smith, C. A. *et al.* *Nature* 337, 181–184 (1989).

The making of modern chemistry

George B. Kauffman

Two hundred years ago Lavoisier's *Traité élémentaire de chimie* was published in Paris. The book stands alongside Newton's *Principia* as a landmark work in science.

"CHEMISTRY is a French science. It was founded by Lavoisier of immortal fame"¹. Although many would argue with the French chemist Charles-Adolphe Wurtz's chauvinistic, exaggerated characterization of chemistry, few would dispute his characterization of Antoine-Laurent Lavoisier (1743–1794) as the founder of the subject in its modern form. Lavoisier himself was aware of the revolutionary nature of his work. On February 20, 1773, in opening a new research notebook — the first of his celebrated *registres de laboratoire* — he announced:

Before commencing the long series of experiments that I propose to make on the elastic fluid which is released from bodies, whether by fermentation, or distillation, or finally by every combination, as well as on the air absorbed in the combustion of a great number of substances, I believe that I ought to put some reflections here in writing, in order to shape for myself the plan which I must follow. . . . The importance of the objective impels me to repeat all that to me seems suitable to bring about a revolution in chemistry and physics².

The spread of Lavoisier's revolutionary doctrines was helped by his classic *Traité élémentaire de chimie*³, which was published two hundred years ago and represents the culmination of his life's work. When Lavoisier began chemical research, the aristotelian four-element theory was still widely accepted. According to the theory, all substances were composed of four elements — earth, air, fire and water — in varying proportions, and could be transmuted into one another by changing the proportions. In his *Sceptical Chymist* (1661) Robert Boyle had criticized both this theory and the three-principle (mercury, sulphur and salt) theory of the Arabs and Paracelsus; Boyle suggested that an element was a substance that could not be decomposed any further, but his views were not widely held.

Chemistry was first systematically ordered in the late seventeenth and early eighteenth centuries by Georg Ernst Stahl, who developed the phlogiston theory from his teacher Johann Joachim Becher's concept of 'fatty earth', the element of inflammability. Unlike the four-element and three-principle theories, this theory, which held sway until the ideas embodied in the *Traité* were accepted, explained not only chemical composition but also chemical reactions and processes. All combustible substances were considered to contain the hypothetical princi-

ple of phlogiston, the word being derived from the aristotelian term for fire element. Theoretically, phlogiston was released during combustion in the form of fire, flame and sometimes light. The same idea was applied to the process of calcination (or rusting) of metals:

Phlogiston

Metal – phlogiston = calx

Calx + phlogiston = metal

Lavoisier

Metal + oxygen = metal oxide
(oxidation)

Metal oxide – oxygen = metal
(reduction)

On 1 November 1772 Lavoisier deposited with the Académie des Sciences a sealed note (*pli cacheté*) describing his initial experiments on combustion, the first steps towards his 'revolution in chemistry'. In it he reported that, on burning, sulphur and phosphorus did not lose weight (as might be expected from a loss of phlogiston), but instead increased in weight because they absorbed "a prodigious quantity of air"; while litharge (PbO), in forming lead by reduction with charcoal, did not gain weight (as might be expected from a gain of phlogiston) but decreased in weight because it had lost "air". He did not explain the exact nature of the "airs". It was only after Joseph Priestley's discovery of "dephlogisticated air" (oxygen) in 1774 that Lavoisier realized that in combustion and calcination only a portion of the common air was used up; he decided that Priestley's newly

discovered "air" was what was absorbed ("vital air"), and that "nonvital air" or "azote" (nitrogen) was what remained. Lavoisier concluded that air was a mixture of two different "elastic fluids" — one that supported combustion and respiration (the "salubrious part") and one that did not (*Mém. Acad. R. Sci.* 351–367, 1774). He later called the active part of the air "eminently respirable air", and he demonstrated that "fixed air" (CO₂) was a compound of charcoal with this air (*ibid.* 520–526; 1775).

In a series of memoirs Lavoisier showed that "eminently respirable air" was contained in phosphoric (H₃PO₄), vitriolic (H₂SO₄) and nitrous (modern, nitric, HNO₃) acids, and that it combined with non-metals to form acids. He therefore renamed it *principe acidifiant* or *principe oxygène* from the Greek terms for 'acid' and 'to beget' (*ibid.* 535–547; 1778); this name held even after 1810, when Sir Humphry Davy showed that hydrogen, not oxygen, was the essential constituent of acids. During the winter of 1782–1783, Lavoisier, together with the mathematician Pierre-Simon de Laplace, invented the first ice calorimeter, with which they measured the heat generated in various chemical and physiological reactions, thus establishing the fields of thermochemistry and physiological chemistry. This research led them to conclude that respiration was a kind of combustion. Lavoisier now began to attack the phlogiston theory (*ibid.* 592–600; 1777) and maintained that combustion and calcination were combi-



Lavoisier at work investigating the phenomenon of respiration. The scene was depicted by his wife, a talented woman, who also drew the copperplate illustrations which adorn the *Traité*.

nations with oxygen rather than decompositions (loss of phlogiston).

In 1783 the Englishman Henry Cavendish obtained water by burning "inflammable air" (hydrogen) in common air and in "dephlogisticated air", but he assumed that the water was originally present as a constituent of both "airs". Lavoisier repeated Cavendish's experiment quantitatively, and on 12 November 1783 he made the historic announcement to the Académie des Sciences that water, previously considered one of the four elements, was a compound of "dephlogisticated air" and the aqueous inflammable principle (*principe inflammable aqueux*) (*Observations sur la physique* 23, 452–455; 1783). This work led to the experiments on the combustion of alcohol and other organic compounds in oxygen, which represent the beginnings of quantitative organic analysis.

Lavoisier's new antiphlogistic theory of combustion slowly gained adherents. Beginning in 1782 Louis Bernard Guyton de Morveau, Claude Louis Berthollet, Antoine François de Fourcroy and Lavoisier collaborated in devising a new system of nomenclature, based on the new chemistry, which led in 1787 to the *Méthode de nomenclature chimique*⁴. This influential book listed provisionally as elements 55 substances that had not been decomposed (*substances non décomposées*). The system marked a complete break with the past and replaced the old fanciful names such as butter of antimony, oil of tartar, spirit of hartshorn and so on by new ones that corresponded to their chemical composition. With some modifications, the new names form the basis for our modern system of nomenclature.

The *Traité élémentaire de chimie* grew out of the *Méthode* and was published in March 1789. It was neither a general reference work nor a technical monograph, but a book for beginners which summarized the discoveries of Lavoisier and his co-workers and which introduced the new chemistry. In the preface Lavoisier described his pedagogical ideas on the learning of science, the new nomenclature and his reliance on experiment:

We must trust to nothing but facts: These are presented to us by Nature, and cannot deceive. We ought, in every instance, to submit our reasoning to the test of experiment, and never to search for truth but by the natural road of experiment and observation.

He rejected the four aristotelian elements and the three paracelsian principles, and he admitted the provisional nature of his own table of elements, which he called *substances simples*:

We cannot be certain that what we regard today as simple is really so; all that we can say is that such a substance is the actual limit reached by chemical analysis, and that, in the present state of our knowledge, it cannot be further subdivided.

The first edition of the *Traité* was published in two volumes, continuously paginated and divided into three parts. In Lavoisier's own words:

In the first part of my work, I make very little use of any experiments but those which were made by myself. . . . The second part is composed chiefly of tables of the nomenclature of the neutral salts. To these I have only added general explanations, the object of which was to point out the most simple processes for obtaining the different kinds of known acids. This part contains nothing which I can call my own, and presents only a very short abridgement of the results of these processes, extracted from the works of different authors. . . . In the third part, I have given a description, in detail, of all the operations connected with modern chemistry. . . . this part could not be borrowed

TABLEAU DES SUBSTANCES SIMPLES.

	NOMS NOUVEAUX.	NOMS ANCIENS CORRESPONDANTS.
	Lumière.	Lumière.
	Calorique.	Chaleur.
		Principe de la chaleur.
		Fluide igné.
Substances simples qui appartiennent aux trois règnes, et qu'on peut regarder comme les éléments des corps.		Feu.
		Matériau du feu et de la chaleur.
	Oxygène.	Air déphlogistié.
		Air empuisé.
		Air vital.
		Base de l'air vital.
		Gas phlogistique.
	Azote.	Mofette.
		Base de la mofette.
		Gas inflammable.
	Hydrogène.	Base du gas inflammable.
		Soufre.
Substances simples, non métalliques, oxydables et acidifiables.	Phosphore.	Phosphore.
	Carbone.	Charbon pur.
	Radical muriatique.	Inconnu.
	Radical fluorique.	Inconnu.
	Radical boracique.	Inconnu.
	Antimoine.	Antimoine.
	Argent.	Argent.
	Arsenic.	Arsenic.
	Bismuth.	Bismuth.
	Cobalt.	Cobalt.
	Cuivre.	Cuivre.
	Étain.	Étain.
Substances simples, métalliques, oxydables et acidifiables.	Fer.	Fer.
	Manganèse.	Manganèse.
	Mercur.	Mercur.
	Molybdène.	Molybdène.
	Nickel.	Nickel.
	Or.	Or.
	Platine.	Platine.
	Plomb.	Plomb.
	Tungstène.	Tungstène.
	Zinc.	Zinc.
Substances simples, solubles, terreuses.	Chaux.	Terre calcaire, chaux.
	Magnésie.	Magnésie, base de sel d'Epsom.
	Baryte.	Barote, terre pesante.
	Alumine.	Argile, terre de l'alun, base de l'alun.
	Silice.	Terre siliceuse, terre vitrifiable.

The table of elements published in the *Traité*, here reproduced from the reprint of 1864.

from any other work, and . . . in the principal articles it contains, I could not derive assistance from anything but the experiments which I have made myself.

In Chapter XIII (Part I) Lavoisier reported a hitherto unpublished quantitative experiment, stating most clearly the law of conservation of matter during chemical reactions:

Nothing is created either in the operations of the laboratory, or in those of nature, and one can affirm as an axiom that, in every operation, there is an equal quantity of matter before and after the operation; that the quality and quantity of the principles are the same, and that there are only alterations and modifications. On this axiom is founded the whole art of making experiments in chemistry: we must suppose in all of them a true equality or equation between the principles of the body one examines, and those that we extract by analysis.

Actually, the idea that matter is neither created nor destroyed originated with the Greek atomists and was commonly assumed by eighteenth-century scientists.

Lavoisier, however, was the first to apply it specifically to chemical reactions. The historian of chemistry, John Riddick Partington, also considered this the first use of the word 'equation' in the sense of chemical equation. In Lavoisier's words, "must of grapes = carbonic acid + alcohol". Ironically, in this case Lavoisier's data contained considerable errors, yet they largely cancelled each other out and yielded a result close to equality.

Part II, the shortest part, includes a "Table of Simple Substances", a modified version of the *Méthode de nomenclature chimique* and the first modern list of the chemical elements. Part III, almost half of the book, deals with a wide variety of techniques and apparatus. Altogether the *Traité* is a truly modern work — in Douglas McKie's words, "Lavoisier had done for chemistry what Newton had done for mechanics a century earlier in his *Principia*".

As a political liberal who sympathized with many of the criticisms against the *ancien régime*, Lavoisier took part in the events leading to the French Revolution. Nevertheless, he was attacked by the radical politician and journalist Jean-Paul Marat. In 1768, Lavoisier had become a member of the Ferme Générale, the government's primary tax-collecting agency, and during the Reign of Terror, the Revolutionary Convention passed a decree on 24 November 1793 ordering the arrest of the former members of the agency (which had been abolished in 1791). On the morning of 8 May 1794, together with 27 of his former colleagues, including his father-in-law, Lavoisier was tried and convicted by the Revolutionary Tribunal. All were guillotined that very afternoon. The widely circulated remark, "The Republic has no need of scientists; let Justice take its course!", attributed to Jean Coffinhal, president of the Tribunal, is apocryphal. But the comment made the following day by Joseph Louis Lagrange is authentic — "It took them only an instant to cut off that head, and a hundred years may not produce another like it". □

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- Wurtz, C.-A. in *Dictionnaire de chimie pure et appliquée* (Hachette, Paris, 1868).
- Berthollet, M. in *La révolution chimique: Lavoisier* 46–49 (Félix-Alcan, Paris, 1890).
- Lavoisier, A.-L. *Traité élémentaire de chimie, présenté dans un ordre nouveau et d'après les découvertes modernes* (Cuchet, Paris, 1789). Translated into English by R. Kerr as *Elements of Chemistry, in a New Systematic Order, Containing All the Modern Discoveries* (William Creech, Edinburgh, 1790).
- Méthode de nomenclature chimique, proposée par MM. de Morveau, Lavoisier, Berthollet [sic] et de Fourcroy. On y a joint un nouveau système de caractères chimiques, adaptés à cette nomenclature, par MM. Hassenfratz & Adet* (Cuchet, Paris, 1787). Translated into English by J. St. John as *Method of Chymical Nomenclature, proposed by Messrs. De Morveau, Lavoisier, Berthollet and Fourcroy, to which is added a new system of chymical characters, adapted to the nomenclature by Messrs. Hassenfratz and Adet* (G. Kearsley, London, 1788).

What to say about cold fusion

Public interest in recent excitements is to be welcomed, especially if it does not turn to anger when attempts to replicate the observation of cold fusion fail — the most probable outcome.

FLEISCHMANN and Pons have done at least one great service for the common cause: they have kindled public curiosity in science to a degree unknown since the Apollo landings on the Moon. In the past few weeks, people thought by those about them to have a few scraps of inside knowledge have been quizzed about the smallest details of the experiments at the University of Utah — those first described in the *Wall Street Journal* and the *Financial Times* on 23 March and at a press conference at Salt Lake City later that same day. The social phenomenon is all the more remarkable because of the wide distribution of those who have a general understanding of nuclear fusion and of the reasons why its practical application would be important.

These impromptu conversations seem generally to be remarkably good-humoured. Those who want to know more, and who in particular ask "Do you think it's true?", seem not too much annoyed by the prospect of continued suspense. It is remarkable that so many people are willing to accept that experimental observations, and the inferences drawn from them, acquire validity only by replication. Has what used to be called "the scientific method" now become widely understood?

If so, much of the credit should go to the daily press, which has risen superbly to the challenge of cold fusion. The two financial newspapers that broke the news on 23 March did so in cautious language, making it plain that cold fusion was not then a proven reality, let alone a commercial source of limitless energy. Other daily newspapers have joined in with commendable zeal and sobriety. Inevitably, the US daily press, with its resources and self-discipline, has done best, but newspapers such as the London *Daily Telegraph*, with no great tradition in the field of science reporting, have been magnificent. Reporters have given full and coherent accounts of meetings as far apart as Sicily and Texas, have canvassed opinions with care and have also faithfully reflected the good humour of these hectic weeks.

The good humour may not persist if the experiments cannot be replicated but there are some grounds for optimism. Part of the reason why so many people are so interested seems to be the general delight that a couple of people in widely separated universities have used their own money to pull off a trick on which governments have

lavished huge sums of money in the past 30 years, so far without result. So people may well be indulgent if the attempts to replicate cold fusion on the scale described by Fleischmann and Pons prove failures. "It was a brave venture", they may say, "What a pity it did not succeed!"

That would be the best outcome. It is also, of course, possible that the general reaction to the failure of attempts at replication will be more sour. The scientific community's reputation is vulnerable in several respects, not the least of which is that neither the Utah group nor the Brigham Young group (whose account of its work appears on page 737, this issue) had, before seeking publication, carried out the rudimentary control experiment of running their electrolytic cells with ordinary rather than heavy water.

A close friend who is a Soviet biologist, on the telephone from the United States last week, was indignant at this neglect. He is correct. How is this astonishing oversight to be explained to students repeatedly being drilled in the need that control experiments should be as conspicuous in the design of an investigation as those believed to display the phenomenon under study? And how should the neglect be explained to the world at large?

There is no convincing explanation, only extenuating circumstances. Self-imposed secrecy has evidently hampered the investigators, understandably buoyed up by their belief that they had discovered a remarkable new phenomenon and fearful that too much talk about it would give other bigger battalions a chance to steal a march on them. Yet it is unthinkable that, if the authors had felt able from the outset to stand in front of routine laboratory colloquia and give a full account of their work, the question "Have you tried it with ordinary water?" would not have been raised. This glaring lapse from accepted practice is another casualty of people's need to be first with reports of discovery and with the patents that follow.

More subtle doubts perplexing those seeking to replicate the experiments would have been exorcised in the same process. The Brigham Young group, for example, clearly explains how it has been necessary to estimate the number of neutrons emitted from its electrolytic cells by subtracting from its observations of neutrons the background measurements, but Dr John M. Carpenter (one of the referees of the article by Jones *et al.*)

explains on page 711 that the background may fluctuate with time, which argues for the need for contemporaneous controls. Sadly, the neutron monitor is a special device, of which there is only one copy....

The Fleischmann and Pons experiments raise bigger questions, if only because the scale of the phenomenon they report is so much greater. Both groups report the detection of neutrons, but the Utah group (*J. Electroanal. Chem.* **261**, 301; 1989) requires that there should be 10^{12} fusion reactions a second (within an order of magnitude in either direction) to account for the rate at which heat is produced, while the Brigham Young group is talking of one fusion reaction every 100 seconds or so. But the Utah group has not yet dealt with the natural question whether the observed energy output is energy stored in the palladium electrodes during the preparation of the cell. Records of cell voltage and current for the full duration of each run would go some way to settle the question, but have not been produced.

So are the extenuating circumstances sufficient to avoid the conviction of the scientific community for irresponsibility? No doubt the general opinion will depend on the outcome of attempts at replication, but the community might wish that its reputation did not hang on such a narrow thread, especially because the likelihood of replication fades as the days go by.

So robust scepticism is the only wise view. There may be something in the Brigham Young phenomenon, but that requires careful confirmation. The Utah phenomenon is literally unsupported by the evidence, could be an artefact and, given its improbability, is most likely to be one.

Luckily, there are a few blessings to count. Theoreticians have zealously recalculated the fusion rate between deuterons in a molecule (now reduced by 10 orders of magnitude) and have shown that the fusion rate between protons and deuterons, against naive expectation, is likely to be six orders of magnitude greater still — but still no more than 10^{-54} per molecule of HD per second. A week or so ago, ingenious schemes for bringing bare hydrogen nuclei more closely together in the electron sea of a palladium lattice seemed to offer an escape from scepticism. But not for long, even if it is plain that metal hydrides are an even more interesting field of research than had been thought.

John Maddox

The descent of the larynx?

John C. Marshall

AMONG the uninitiated the discovery of an old human hyoid bone reported by Arensburg *et al.* on page 758–760 of this issue¹ is likely to raise three questions: what is it; where is it; and why should anyone care? The answer to the first question is a small U-shaped bone, consisting of a body with two greater and two lesser horns. Between the root of the tongue and the larynx is the answer to the

It is at this point that the hyoid bone makes contact with a long-running controversy about the evolution of speech. Extant primates (monkeys and apes) do not spontaneously produce the range of phonetic vocalizations characteristic of human speech, and attempts to teach them to do so have met with very limited success². Does their 'failure' reflect differences in the anatomical configuration of

their vocal tracts, or is it rather the case that they lack the requisite neuronal structures for the control of articulate speech? Computer-implemented modelling of the supralaryngeal vocal tract seems to indicate that some, but not all, vowels and consonants are "virtually impossible for the chimpanzee to articulate" (ref. 3).

These simulations by Lieberman, Crelin and Klatt³ have not, however, met with unqualified acceptance. For example, the point vowels *i*, *u* and *a* cannot be simulated by their reconstruction of the chimpanzee vocal tract,

what degenerate specimen, deformed in life by arthritis, and after death by the very process of fossilization. Moreover, his hyoid bone is missing. As Lieberman⁵ himself writes in a later discussion of the problems that arise when one tries to infer speech and language capacities from a fossil: "Nothing remains of the brain, the soft tissue of the vocal tract, or small bones like the hyoid and the cartilages of the larynx".

One of these statements has now been falsified. Arensburg *et al.*¹ have recovered a well-preserved human hyoid bone from Middle Palaeolithic layers of Kebara Cave, Mount Carmel, Israel. The specimen dates from approximately 60,000 years BC. Furthermore, the size and shape of this hyoid, and the positioning of the marks left by muscle attachment, are, for the most part, within the range of modern man. It is well known that the middle ear ossicles of Palaeolithic Neanderthals are likewise very similar to our own. Arensburg *et al.*¹ accordingly hypothesize that "the osseous elements derived from the embryonic branchial arches show less rapid evolutionary changes than bones derived from dermal or enchondral tissues". And they boldly speculate that the larynx itself may not have altered significantly since the Middle Palaeolithic.

Their evidence about the hyoid certainly makes it more likely that Neanderthal man was morphologically adapted to speech, although they do not elaborate in their paper on the hypothetical positioning of either the hyoid or the larynx. The inference that the Neanderthal larynx was positioned too high in the throat to produce *i*, *u* and *a* was a crucial feature of Lieberman's model⁵. It is generally accepted that the larynx descended in two stages and that in the first stage only neutral vowels could be formed. Consistent with this claim, Kratz⁶ reports that

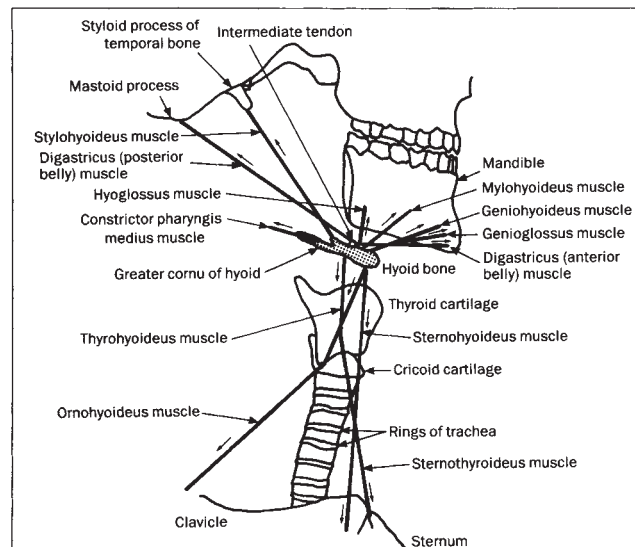


FIG. 1 The extrinsic laryngeal musculature showing the direction of movement of the hyoid bone, when the muscles contract from fixed origins. (From ref. 12.)

second. The third question is, of course, the interesting one.

In addition to supporting the base of the tongue, the hyoid bone is connected to the cartilaginous framework of the larynx by eleven muscles. Accordingly, any movement of the hyoid affects the positioning and movement of the larynx (Fig. 1). The extrinsic muscles of the larynx form two groups; the suprahyoid muscles, which act as laryngeal elevators; and the infrahyoid, which act as laryngeal depressors. These changes in turn affect the configuration of the supralaryngeal vocal tract and hence the nature of the noises produced when quasi-periodic bursts of air from the larynx are released through the oral and nasal cavities. Thus vigorous raising of the larynx decreases the volume of the supra-glottal cavity and thereby increases the supra-glottal pressure, to an extent permitting the production of such ejective sounds as *p'* or *t'*. Similarly, when the anterior belly of the digastric muscle contracts from a fixed mandible (lower jaw), the hyoid, and hence the tongue, will move anteriorly and upward. The movement is required for such alveolar articulations as *s* or *i*. In short, then, the humble hyoid is vitally implicated in the mechanics of human speech production.

yet Jordan⁴ reports that (real, live) chimpanzees do produce at least two of them! Lieberman and colleagues also claim, on the basis of a reconstruction of the La Chapelle-aux-Saints fossil, that Neanderthal man could not produce the point vowels. This skull, however, is a some-

IMAGE
UNAVAILABLE
FOR COPYRIGHT
REASONS

FIG. 2 Conversation piece? An artist's impression of a Neanderthal family, by Maurice Wilson.

"linguistic reconstruction of the pre-proto-Indo-European language indicates an absence of differentiated vowels", although he dates this prehistoric language from a mere 10,000 years ago.

With respect, however, to the fossil record, it must be stressed that the distance from morphology to function is considerable. Who would have guessed solely from consideration of the anatomy of the syrinx — a structure very different from the larynx — that the mynah bird could produce such a passable imitation of human speech? Who, for that matter, would suspect that humans can learn to produce intelligible speech after surgical removal of the tongue, or of the larynx? Severe deformity of the vocal tract is compatible with human speech if a human brain is in command thereof. There are some lateral asymmetries of the modern brain that leave an impression on the skull, and these can be found on endocasts of Neanderthal man¹⁰. Such asymmetries at the posterior end of the sylvian fissure are thought to be related to linguistic capacity, although they can also be seen in apes that most emphatically do not possess language abilities.

The best witness to Neanderthals' intellectual level can perhaps be found in reconstructions of their culture (Fig. 2). There is good evidence of advanced stone tool manufacture, and presumptive evidence that Neanderthals "buried their dead with symbolic artifacts, including red ochre, animal bones, and flowers"¹¹. The possibility that Neanderthals engaged in ritual murder¹² also links them to modern man, although whether one wants to regard this as an example of 'high culture' is, I suppose, debatable. All our inferences about the language, thought and culture of Neanderthals are based upon long, fragile and indirect lines of reasoning. The argument about their language capacity will undoubtedly run and run until we discover a deep-frozen Neanderthal who is susceptible to resuscitation. □

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Mechanisms of phase changes

D. C. Rubie

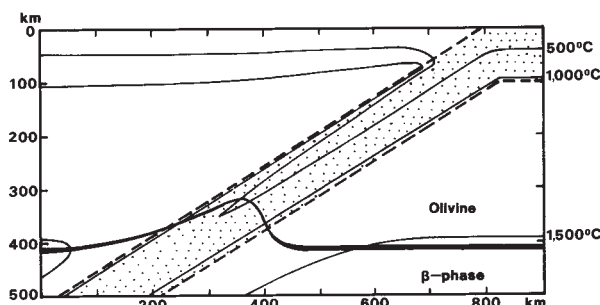
THE most abundant phase in the Earth's upper mantle is orthorhombic (Mg,Fe)₂SiO₄ olivine. With increasing pressure, this phase transforms sequentially to the higher-density polymorphs β -phase (modified spinel structure) and γ -spinel. The transformation of olivine to β -phase coincides with and is generally believed to be responsible for the seismic discontinuity observed at a depth of 400 km. The mechanism of this phase transition, which is important in discussions of subduction dynamics, mechanisms of deep-focus earthquakes and the rheology of subducting lithosphere, has been a subject of debate for over 10 years. The new results of Burnley and Green reported on page 753 of this issue¹ now show without doubt that olivine can transform to the high-pressure spinel structure by two different reaction mechanisms, depending on the magnitude of differential stress as well as

mantle (see figure). The density of β -phase is about 8 per cent greater than that of olivine, and thus an elevated phase boundary such as that shown in the figure would produce a large negative buoyancy force which would contribute significantly to the driving forces of subduction and mantle convection^{2,3}.

But if temperatures in the subducting slab are as low as suggested by thermal modelling (500–600 °C at a depth of 300 km), the kinetics of the transformation may become important in determining the position of the phase boundary. In this case, olivine persists metastably in a subducting slab, the elevation of the phase boundary and the negative buoyancy force are less than that predicted by an equilibrium model, and the rate of subduction may be controlled to a large extent by the kinetics of the transformation^{4,5}. For this hypothesis to be

tested, experimental kinetic data must be extrapolated to subduction-zone conditions — a virtually impossible task unless the mechanism of transformation is understood so that an appropriate rate law can be used.

In experimental investigations of the transformation mechanism, compounds such as Mg₂GeO₄, Ni₂SiO₄ and Co₂SiO₄ have often been used because their high-pressure polymorphs are formed at pressures



Thermal structure of a subduction zone based on numerical modelling. The subducting slab (stippled) is bounded by dashed lines and 500 °C, 1,000 °C and 1,500 °C isotherms are shown (solid lines). The depression of the 500 °C isotherm to a depth of about 350 km in the slab causes the olivine- β -phase equilibrium boundary (thick line) to be elevated in the slab ~ 100 km relative to the boundary in the adjacent mantle. (After Helffrich *et al.*¹⁰.)

the pressure-temperature conditions.

The nature of the transformation mechanism is particularly important in discussions of the driving forces of plate tectonics. Oceanic plates are created at mid-ocean ridges by the process of sea-floor spreading. The complementary process of plate consumption occurs at ocean trenches where two plates collide and one of the plates descends deep into the mantle by a process known as subduction. The driving force for subduction comes from the density contrast between the cold slab and the hot surrounding mantle (see figure). In addition, a large driving force may result from density increases produced by high-pressure phase transitions such as olivine to β -phase or spinel. The pressure-temperature slope (dP/dT) of the olivine- β -phase equilibrium boundary is positive so that in subduction zones the position of the equilibrium boundary between the two phases is elevated by as much as 100 km relative to the adjacent

which are readily attainable experimentally (1–6 gigapascal). Phases with these compositions are regarded as analogues of the mantle phases, although only spinel (and not β -phase) occurs as a high-pressure polymorph in Mg₂GeO₄ and Ni₂SiO₄.

One mechanism for the transformation of olivine to spinel or β -phase involves incoherent nucleation (on olivine grain boundaries for example), with subsequent growth controlled by diffusion across the interphase boundary⁴. Another, which is analogous to a martensitic transformation and results from the propagation of partial dislocations, operates by shear of the oxygen sublattice and accompanying 'synchroshear' of the cations⁵. The new results of Burnley and Green¹ on the transformation of olivine to spinel in Mg₂GeO₄ show that either of these mechanisms is possible depending on the experimental conditions. The incoherent nucleation and growth mechanism operates at low differential stress, high temperatures (above

1. Arensburg, B. *et al.* *Nature* **338**, 758–760 (1989).
2. Hayes, C. *The Ape in our House* (Harper, New York, 1952).
3. Lieberman, P., Crellin, E.S. & Klatt, D.H. *Am. Anthropol.* **74**, 287–307 (1972).
4. Jordan, J. *Folia Morph.* **30**, 97–126; 222–248; 323–340 (1971).
5. Lieberman, P. *On the Origins of Language* (Macmillan, New York, 1975).
6. Kratz, G.S. in *The Genesis of Language* (ed. Landsberg, M.E.) 173–180 (de Gruyter, Berlin, 1988).
7. Klatt, D. & Stefanski, R. *J. acoust. Soc. Am.* **55**, 822–832 (1974).
8. Luchsinger, R. & Arnold, G.E. *Voice – Speech – Language* (Constable, London, 1965).
9. LeMay, M. *Am. J. phys. Anthropol.* **42**, 9–14 (1975).
10. LeMay, M. in *Cerebral Dominance* (eds Geschwind, N. & Galaburda, A.M.) 26–42 (Harvard University Press, 1984).
11. Marshack, A. *Hierarchical Evolution of the Human Capacity: The Palaeolithic Evidence* (American Museum of Natural History, New York, 1985).
12. Hardcastle, W.J. *Physiology of Speech Production* (Academic, London, 1976).

700 °C) and conditions relatively close to equilibrium. In contrast, nucleation occurs by the shear mechanism under conditions of high differential stress, moderate temperature (600 °C) and pressure-temperature conditions which deviate significantly from equilibrium.

The different mechanisms observed in earlier experimental studies can now be readily explained on the basis of these new results¹. Evidence for the incoherent nucleation and growth mechanism has come primarily from experiments carried out in large-volume high-pressure apparatus (for example, piston-cylinder and multi-anvil devices), whereas evidence for the shear mechanism has previously been found only in the products of diamond-anvil-cell experiments. The variability of these previous results can therefore be attributed primarily to the level of differential stress in the high-pressure apparatus.

The mechanism of the transformation of olivine to β -phase or spinel both in the mantle and in shocked meteorites can now be considered. At the 400-km discontinuity, where the temperature is high (1,400–1,500 °C) and differential stresses are low, the transformation probably occurs by the incoherent nucleation and growth mechanism. In shocked meteorites, however, olivine is most likely to transform to the high-pressure polymorphs by the shear mechanism under conditions of low temperature and very high differential stress. In subduction zones, where both temperature and differential stress have intermediate values, it is still uncertain which mechanism dominates. Apart from the questions of kinetics and the possible elevation of the phase boundary, there are potentially important implications associated with the two transformation mechanisms.

First the mechanism will influence the post-transformation microstructure and consequently may affect the rheology and mechanical properties of subducting lithosphere. At conditions which deviate significantly from equilibrium, transformation by the incoherent nucleation and growth mechanism results in very fine-grained spinel⁷ (grain size 1–3 μ m). In contrast, preliminary results suggest that when nucleation occurs by the shear mechanism, subsequent growth results in much coarser-grained spinel¹. In the former case, the slab may deform by a grain-size-dependent superplastic mechanism and consequently the strength of the slab would be much lower than in the latter case⁸.

Second, there may be a close relationship between high-pressure phase transformations in subduction zones and the mechanisms of deep-focus earthquakes. Although this idea was first proposed more than 40 years ago, its validity and the nature of such a relationship are both

Fossils from the Miocene of Abu Dhabi

THE Arabian peninsula was a unique land mass through which faunal elements from Europe, Asia and Africa could migrate and mix in the late Tertiary, about 25–2 million years ago. Its fauna at any particular epoch can be used to infer its geographical connections with other parts of the world. Therein lies the importance of a palaeontological expedition to the United Arab Emirates in January which unearthed an important fauna from the Late Miocene (about 8 million years ago). The fauna,

great seaway had been the overriding factor in animal migrations across the Old World since the late Palaeozoic, when it formed the boundary between the ancient land-masses of Laurasia (North America and Eurasia) and Gondwanaland (the southern continents and India). The Arabian Peninsula became an important land bridge: in the Miocene, the Red Sea was open to the Mediterranean, but was closed at its southern end by a land bridge between Ethiopia and the Yemen. Further east, the Tigris-Euphrates river system extended much further south than it does today. The new fauna represents a thriving community from what seems to have been the delta of this system. This idea is supported by the extent of the fauna, known from sites over an area of 16,000 square kilometres.

Apart from the intrinsic value of the fossils, Whybrow's team was able to refine knowledge of the stratigraphy of the Gulf Neogene. One example of this is their discovery of the horse-like *Hipparion* in the sediments, restricting rocks previously designated as 'undifferentiated Tertiary' to an age of less than 11.2 million years. *Hipparion* is not known from the Old World any earlier than this, and is an important zone fossil.

Well-watered palaeoenvironments from this region may seem surprising in the light of conventional wisdom about the Gulf area, long thought to have been arid for an extended period. The discovery of fossil fish in sandstone structures thought to represent dune fields forced the reevaluation of these formations as fluvial. In fact, desert conditions are unknown from the Miocene of this part of the world. The subsequent desiccation of the peninsula (during the Plio-Pleistocene) is related to increasing aridity worldwide: savannah opened up in Africa and the shrinkage of forests led to the development of ground-living hominids as well as modern plains animals such as grazing horses and ruminants. But the great drying was punctuated by short, wet episodes. In the Pleistocene, these may have been in synchrony with the glacial-interglacial cycle of the northern ice ages. Even 8,000 years ago, large parts of Arabia were green and pleasant, and areas now considered barren produced crops well into historical times.

Henry Gee

IMAGE UNAVAILABLE FOR COPYRIGHT REASONS

Canine tooth (about 26 mm high) of a macaque-like monkey found in Abu Dhabi.

discovered by Peter Whybrow of the British Museum (Natural History) and his colleagues, consisted of deinotheres (proboscideans distantly related to modern elephants), hippopotami, small carnivores, horses, rhinoceroses, turtles, crocodilians, fish, birds and a macaque-like monkey (see figure). Ostrich eggshells twice as thick as any known today revive the myth of Sinbad's roc, a mythical bird large enough to carry off full-grown bull elephants. The fauna is clearly of Ethiopian origin, but artiodactyl (possibly cervid) limb bones may betray the presence of a few Holarctic elements from south-west Asia.

By 8 million years ago, the Tethys Ocean that had stretched across the Near and Middle East from the Mediterranean Sea to the Indian Ocean had long since closed: this

still uncertain⁹. The knowledge of the mechanisms of phase transformations in subduction zones should alleviate this uncertainty. □

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1. Burnley, P.C. & Green, H.W. *Nature* **338**, 753–756 (1989).
2. Schubert, G., Yuen, D.A. & Turcotte, D.L. *Geophys. J. R. astr. Soc.* **42**, 705–735 (1975).

3. Christensen, U.R. & Yuen, D.A. *J. geophys. Res.* **90**, 10291–10300 (1985).
4. Sung, C.M. & Burns, R.G. *Tectonophysics* **31**, 1–32 (1976).
5. Bassett, W.A. *Rev. Earth planet. Sci.* **7**, 357–384 (1979).
6. Poirier, J.P. in *Elastic Properties and Related Processes in the Earth's Mantle*, *Geodynamics Ser.* **4**, 113–117 (Am. Geophys. Union, Washington DC, 1981).
7. Rubie, D.C. & Champness, P.E. *Bull. Mineral.* **110**, 471–480 (1987).
8. Rubie, D.C. *Nature* **308**, 505–508 (1984).
9. Kirby, S.H. *J. geophys. Res.* **92**, 13789–13800 (1987).
10. Helffrich, R.H., Stein, S. & Wood, B.J. *J. geophys. Res.* **94**, 753–763 (1989).

The cold fusion family

James S. Cohen and John D. Davies

CLAIMS for the observation of cold fusion are based on two types of evidence. Jones *et al.*, on page 737 of this issue¹, report that they have detected neutrons, with energy characteristic of a fusion reaction, in an electrochemical cell containing deuterium. And Pons and Fleischmann report in the 10 April issue of the *Journal of Electro-analytical Chemistry*² the production of unexplained excess heat and also neutrons in a similar cell. It is wise to see whether the observations can be explained using standard physics before seeking novel solutions — especially as the two reports conflict greatly in detail.

Cold fusion is a known process, but not as described in the new work. A muon — a heavy analogue of the electron — substituted for an electron in a deuterium molecule can cause the molecule to contract so that there is a significant probability of the two nuclei coming into contact. A fusion reaction can follow in which energy is carried away by the products. The muon is released and can induce further fusions. This is muon-catalysed fusion³.

Considerations of this process in the context of the present experiments is natural, because Van Sieten and Jones have previously suggested⁴ that very high pressures could similarly induce fusion; because some have suggested that muons from cosmic rays could be responsible for the observed effects; and because lessons about the relative rates of different fusion reactions could be important. The reactions of interest are those of deuterons ($d=^2\text{H}$), tritons ($t=^3\text{H}$) and protons ($p=^1\text{H}$).

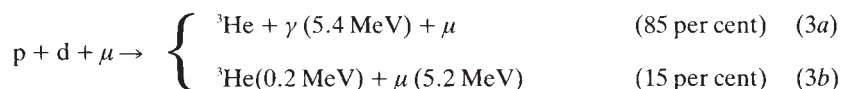
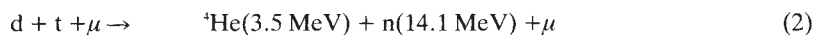
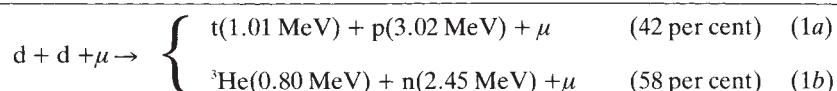
correspondingly. The mean time for a fusion reaction then becomes (ref. 5) 8×10^{-11} s for d-t, 2×10^{-9} s for d-d. (The lesser speed of the d-d reaction arises in part because fusion occurs in a state with one unit of angular momentum which creates a centrifugal barrier to fusion. The d-t molecule is also initially formed in such a state but, because of its asymmetry, rapidly makes a transition to a zero-angular-momentum state.)

It is useful to consider the description of fusion in nuclear-scattering experiments. Besides the probability of barrier penetration, the subsequent interaction between the nuclei (expressed in the 'astrophysical' S function) has an important influence on the fusion rate or cross-section σ :

$$\sigma = \frac{S}{E} \exp(-31.3 \sqrt{\mu E})$$

where μ is the 'reduced' mass in atomic mass units of the colliding nuclei and E is the centre-of-mass collision energy in keV. The variation of S for the various reacting nuclei can be crucial. In the low-energy limit, which clearly applies to cold fusion, $S = S_0$ and is smallest for the p-d reaction (2.5×10^{-4} keV barns)⁶: this is because the final stage involves the emission of a γ -ray, an electromagnetic process which is inherently weaker than the strong-force interactions of the other fusion reactions. For d-d, S_0 is 1.08×10^2 (including both channels⁷); for d-t, which involves a strong resonant intermediate state of ^3He , S_0 is 1.15×10^4 (ref. 8).

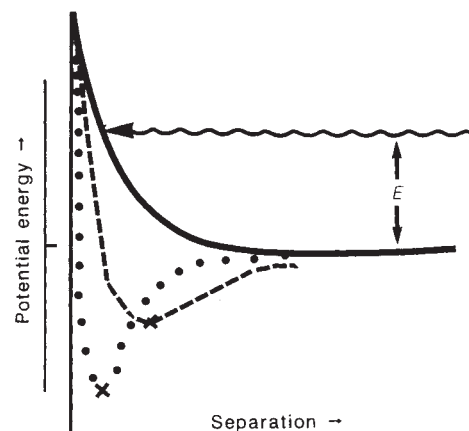
Nevertheless, at low energy the



Here n signifies neutrons; γ , γ -rays; μ , negative muons; the energy carried by fragments is given in mega-electron volts; and the percentages signify branching fractions in muon-catalysed reactions.

Before fusion, the nuclei are held apart by Coulomb repulsion. This barrier is penetrated by quantum-mechanical tunnelling, rather than being surmounted. Note that tunnelling is controlled more by the width of the barrier than by its height. Thus, replacing the electron in a hydrogen ion by a muon reduces the separation of the nuclei by a factor almost equal to the muon/electron mass ratio (207), reducing the 'tail' of the Coulomb barrier

'Gamow' penetrability factor becomes dominant, and significant variations arise between the various fusion rates because of the variation in reduced mass. Thus for bare nuclei, the d-t fusion rate exceeds that of d-d for collision energies above about 400 eV, and the p-d rate exceeds that of d-d below about 200 eV. The implications of the two factors for the case of cold fusion, in which the Coulomb repulsion is shielded by electron or muon screening, is also instructive. Only the barrier penetrability factor is changed by the screening (see figure); also we have just established some useful energies at which the relative fusion rates change.



Schematic of the potentials between two nuclei (not to scale): solid line, unscreened; broken, with electron screening (chemical bonding); dotted, with muon screening. To the right of the barrier, electrostatic interactions are dominant; to the far left, strong nuclear interactions occur as expressed by the astrophysical S factor. Wavy line indicates collision energy E in scattering experiments, the classical distance of closest approach (R_0) at the arrow head. The bond length (x) is actually about 207 times shorter for muons than for electrons and the potential well is about 207 times deeper.

From these it is possible to show that muon screening favours d-t fusion; normal electron screening favours p-d fusion (although in this case fusion rates are unmeasurably slow).

The characteristic separation in the isotopic hydrogen molecules is the bond length — normally somewhat greater than the distance R_0 at which repulsion between the nuclei becomes apparent. Allowing the nuclei easily to approach this close is roughly equivalent to colliding the bare nuclei together with a centre-of-mass energy $14/R_0$ eV where R_0 is measured in ångströms. (Such simple considerations have to do for now since an accurate calculation of the penetrability requires knowledge of the potential energy interaction of d-d in the crystal, which is unknown for electrochemical cold fusion.) For the ordinary hydrogen molecule, $R_0 = 0.7$ Å, so that the equivalent energy is about 20 eV, well below the value 400 eV at which d-t fusion becomes faster than d-d. But with the ion's electron substituted by a muon, $R_0 = 4 \times 10^{-3}$ Å, so that the equivalent energy is about 3,500 eV and d-t fusion is clearly favoured (by a factor of 36 in the zero-angular-momentum state according to calculations of C.-Y. Hu (personal communication)). Although muon-catalysed fusion presents the most dramatic evidence of the effect of screening on fusion rates, similar electron-screening effects have been seen in reactions such as (ref. 9) $^3\text{He} + d \rightarrow ^4\text{He} + p$.

Screening effects should be considered in discussing the new results. Any means of reducing R_0 , vibrational excitation of the bond, a reduction in the bond length, or even a flattening of the potential-

energy well could be effective. It is interesting to note that for the shielding distance relevant to the new studies (of the order of 10^{-1} Å) the p-d fusion rate should exceed that for d-d. This reaction should be studied experimentally; there seems to be no advantage in using d-t, however.

Some have suggested that the new results could be an artefact due to fusion catalysed by cosmic-ray muons. This seems most improbable: the muon stopping flux is not high; the fraction captured by deuterium is low; and the fraction retained by deuterium is even lower. Also, because the muonic molecule formation rate is too slow and because a significant fraction of the muons stick to the helium at fusion, each one can catalyse only a few reactions.

It is reasonable to take $0.1 \text{ g}^{-1} \text{ h}^{-1}$ to be the upper limit to the rate for muons being stopped in a Utah basement (J. Osborne, personal communication). Heavy elements are better at stopping muons, the capture ratio being roughly proportional to nuclear charge Z (ref. 10). Moreover, any $d\mu$ would initially have about an electron volt of kinetic energy¹¹: while slowing down, many muons would be transferred to the high- Z atoms¹⁰ (high- Z contaminants are kept to less than 10^{-4} of contents in muon-catalysed fusion experiments). Lastly, the time for formation of the muonic deuterium molecule is comparable to the muon lifetime ($2.16 \mu\text{s}$) so that few fusions can be catalysed by each muon.

For the sake of argument, however, assume that the above limitations can be removed: that is suppose that channelling in the crystalline electrodes keeps muons from the high- Z atoms and the deuterium density is so high that d-d collision rates do not limit the process. The lifetime of a muon would then be sufficient to catalyse 1,000 fusions. But this also assumes it is continuously available for catalysis. In fact, in 8 per cent of d-d fusions the muon sticks to the ^3He to form muonic helium¹². Although in subsequent collisions of the muonic helium with deuterium, the muon could be stripped this will happen for only 32 per cent of captured muons, even with very high deuterium density¹³. (The high- Z elements have large stripping cross sections, but stripping in this case often results in capture of the muon and so is not helpful¹⁴.) Hence, even under the most favourable circumstances, a single muon would catalyse only about 20 d-d fusions (or 340 d-t fusions). With the above muon stopping rate and assuming an electrode mass of 3 g, the neutron production rate would be $2 \times 10^{-3} \text{ s}^{-1}$, well short of the 0.41 s^{-1} reported by Jones *et al.*¹.

Of course, if the fusion is catalysed by some stable charged quasiparticle in the electrode, one no longer has to contend with the short lifetime encountered with the muon. This can even offset the slower fusion rate that is inevitable with quasi-



Fusion in 1926: *plus ça change*

ON September 17, the *Morning Post* published a Reuter message from Berlin to the effect that Profs. Paneth and Peters of that city had, after years of experimenting, succeeded in transforming hydrogen into helium "with the aid of particles of metal." This announcement, if correct, is of great importance and will evoke even more interest than the claim by Miethe and Stammreich to have transmuted mercury into gold.

No particulars are yet to hand concerning the methods adopted by Profs. Paneth and Peters, for the statement "with the aid of particles of metal" is meaningless as it stands. The experimental difficulties must be very great, not only in obtaining the energy necessary for such a change, but also in applying it under the appropriate conditions. Moreover, helium is an atmospheric gas, and traces of it are difficult to eliminate by the methods of evacuation and adsorption at present in use; belief or disbelief in the Reuter message must be reserved pending further and more definite evidence.

* * *

PROFS. F. Paneth and K. Peters describe in outline how they have succeeded in detecting the presence of very minute amounts of helium, of the order of one hundred millionth of a cubic centimetre, derived from hydrogen which had been absorbed by finely divided palladium at the ordinary temperature.

Finely-divided palladium, either as sponge, 'block' or palladinised asbestos, was used to absorb hydrogen at room temperature. The residual gas obtained after a 12-hours' contact between palladium and hydrogen exhibited four or five lines of the helium spectrum; there was also a distinct proportionality between the amount of helium observed and

the duration of the time of contact. The activity of the different palladium preparations employed varied considerably; it invariably diminished with repeated use. No helium production was observed with palladium preparations that did not absorb hydrogen. They were not able to detect any trace of the energy liberated during the transformation, and they point out that the amount set free from the conversion of such small quantities of hydrogen — about 0.28 calorie — would be extremely difficult to detect, particularly if thermal changes due to absorption or formation of compounds also take place. They incline to the view that the liberated energy is more likely to appear as radiation, γ or Millikan-rays, than as heat.

* * *

A FEW months ago, K. Peters and I published an account of experiments we had made in an attempt to transmute hydrogen into helium (*Ber. d. Deutsches Chem. Ges.*, 59, 2039, 1926). As a result of further experiments, we are in a position to give an explanation of the occurrence of the observed very small quantities of helium in our experiments without having recourse to the assumption of a synthesis of helium.

In the communication we discussed the possibility of regarding the helium dissolved in the glass as an explanation of the observed effects, but blank experiments led us to the conclusion that the quantity of helium liberated in this way was beyond the sensitivity of our method of detection. In the interval we have carried out experiments that show that the liberation of helium from glass is dependent on the presence of hydrogen. Thus glass tubes which gave off no detectable helium when heated in a vacuum or in oxygen yield helium in quantities of the order of 10^{-6} c.c. when heated in an atmosphere of hydrogen.

As a result we have thus established that, in using an apparatus made of glass, one cannot make any trustworthy statement as to the origin of 10^{-9} c.c. of helium if air comes in contact with the apparatus.

From *Nature* **118**, 455 & 556 (1926); **119**, 706 (1927).

particle masses less than that of the muon. Van Siclen and Jones's earlier calculations⁴ suggest that reducing the hydrogen bond length by a factor of 2 (we believe a larger factor is needed), achievable with a quasiparticle with twice the electron mass, might give the neutron production rate reported now by Jones *et al.*¹. Large effective electronic masses are often associated with transition metal crystals like palladium; unfortunately, the notion of heavy electrons is related to the non-local interactions of electrons with the crystal lattice; it is not easy to see how the local concept of tight binding can be squared with this.

The neutron rates observed by Fleischman and Pons² present further difficulties. Not only are they much higher, at about 10^4 s^{-1} , than those of Jones *et al.*, but they are a factor of about 10^3 less than expected from the rate of d-d fusions necessary to generate the heat they report. Thus they suggest some form of aneutronic fusion is occurring: for example a preference for

reaction 1a over 1b.

The equality of rates for 1a and 1b given by charge symmetry of nuclear forces is slightly altered by Coulomb 'isospin' mixing (G. M. Hale, personal communication). For bare nuclei (plasma fusion) the $p + t$ product channel is very slightly favoured, but for muon-catalysed fusion the $n + ^3\text{He}$ channel is favoured by a ratio of 1.4 (ref. 15). The latter is a consequence of fusion taking place in a state of unit angular momentum. In any event, it would seem quite difficult to suppress the neutron channel or to hide the tritium from assay. The normally rare radiative channel, $d + d \rightarrow ^4\text{He} + \gamma$ (20 MeV), would also show up in the lower-energy γ -ray detector from pair production. On the other hand, only heat would be detected if it were somehow possible for the energy to go directly into the palladium crystal lattice ('Mössbauer fusion'?), but such a process is expected to be strongly suppressed by the vastly different energy scales of the crystal lattice (eV) and fusion

ECOLOGY

(MeV). Likewise, as indicated by the small branching fraction for reaction (3b), transfer of all the energy to a heavy electronic quasiparticle, even if it exists, is highly unlikely unless it is even more massive than the muon. Curiously, an excess amount of ^4He did show up in the assay done by Fleischmann and Pons.

The above difficulties in interpreting the Fleischmann and Pons experiment in terms of d-d fusion lead one to consider the possibility of other nuclear reactions that might not produce neutrons or tritons. The electrolyte contained lithium so that $^7\text{Li} + \text{d} \rightarrow ^4\text{He} + ^4\text{He}$ fusion is possible at the electrode surface or with long lithium drift times into the palladium; the intermediate state, ^8Be has a rich set of resonant energy levels to enhance the reaction, but it is difficult to see how the neutronic reactions $^7\text{Li} + \text{d} \rightarrow ^8\text{Be} + \text{n}$ and $^7\text{Li} + \text{d} \rightarrow \text{n} + ^4\text{He} + ^4\text{He}$ could be suppressed. Another difficulty is the smaller penetrability that comes from the greater nuclear charge, the higher reduced mass and the larger bond distance. For similar reasons, a cold fusion of d with Pd can be considered highly unlikely. Here again we note that there is the possibility of reaction with p as well as d, namely $^7\text{Li} + \text{p} \rightarrow \alpha + \alpha$.

In all this discussion, we have presumed that the current observations^{1,2} truly belong to the cold fusion family. We might point out that they could be but a distant cousin. For example, experiments show that d-d fusion neutron emission can accompany the fracture of a LiD crystal¹⁶; the interpretation is that deuterons are accelerated to kiloelectron volt energies by the strong electric fields at the propagating crack. Such an effect might occur in the embrittled Pd or Ti crystal, in which case we would have 'microscopically hot' fusion under very unusual conditions. Neutrons would be emitted in bursts, not continuously. Other means of creating cracks suddenly would lead to similar effects. □

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1. Jones, S. E. et al. *Nature* **338**, 737–740 (1989).
2. Fleischmann, M. & Pons, S. J. *electroanal. Chem.* **261**, 301–308 (1989).
3. Jones, S. E. *Nature* **321**, 127–133 (1986).
4. Van Siclen, C. DeW. & Jones, S. E. *J. Phys.* **G12**, 213–220 (1986).
5. Bogdanova, L. N. *Muon catal. Fusion* **3**, 359 (1988).
6. Griffiths, G. M., Lai, M. & Scarfe, C. D. *Can. J. Phys.* **41**, 724–736 (1963).
7. Brown, R. E. & Jarmie, N. *Phys. Rev. C* (in the press).
8. Brown, R. E., Jarmie, N. & Hale, G. M. *Phys. Rev.* **C35**, 1999–2004 (1987).
9. Engstler, S. et al. *Phys. Lett.* **B202**, 179–184 (1988).
10. Gershtein, S. S. & Ponomarev, L. I. in *Muon Physics Vol 3* (eds Hughes, V. W. & Wu, C. S.) 141–233 (Academic, New York, 1975).
11. Breunlich, W. et al. *PSI Newsletter* (1989).
12. Bogdanova, L. N. et al. *Phys. Lett.* **B161**, 1–4 (1985).
13. Cohen, J. S. *Phys. Rev. Lett.* **58**, 1407–1410 (1987).
14. Cohen, J. S. *Phys. Rev.* **A37**, 2343–2348 (1988).
15. Balin, D. V. et al. *Phys. Lett.* **B141**, 173–176 (1984).
16. Klyuev, V. A. et al. *Sov. tech. Phys. Lett.* **12**, 551 (1986).

Honeyguides and humans

Robert M. May

ONE of my favourite children's books is an engagingly illustrated account of some of the mutualistic associations found in nature. One such illustration is of the honeyguide — appropriately named *Indicator indicator* — perched on a branch over a bee colony being torn apart by a badger-like ratel. The ratel has been led to the hive by the bird, and after the ratel has finished feasting on the honey, the honeyguide will feed on fragments of honeycomb left behind. The honeyguide needs the ratel because the bee colonies are typically situated in large trees, rock crevices or termite mounds in such a way as to be inaccessible to the unaided bird; the ratel apparently benefits from the honeyguide's deliberately leading it to the source of honey. Isack and Reyer¹ have

Boran people in northern Kenya is therefore timely and interesting. Treating this system as if it were any other field study of a mutualistic association, the authors show that, in unfamiliar areas, honey-hunting human groups take on average 3.2 hours to find each bees' nest when guided by a bird, and 8.9 hours when not guided. This roughly threefold reduction in searching efficiency without a honeyguide is a conservative estimate, because the figures do not include the many days of unguided search on which no nest was found.

Isack and Reyer also document the benefit to the birds: 96 per cent of the nests they saw discovered (178 of 186) would not have been accessible to the birds until humans had opened them with tools. In addition, the Boran's use of smoky fire reduces the bird's risk of being stung. Because of the pronounced benefits to both parties, it is not surprising that humans and honeyguides have elaborated upon the previous ratel-honeyguide association by developing their own inter-specific communication system. Humans attract the bird with a penetrating whistle that can be heard more than 1 km away; Isack and Reyer found such whistles doubled the rate at which birds were encountered. On its part, the honeyguide attracts human attention by flying close or hopping around among perches, emitting a characteristic double-noted call.

Once humans and birds are engaged in a cooperative quest, Isack and Reyer show that the bird leads in consistently direct routes to colonies up to 1 km or more distant. Isack and Reyer find quantitative support for Boran honey-collector lore, demonstrating that three measures of bird behaviour decrease with diminishing distance to the bee colony: (1) the length of time the bird disappears after the first encounter; (2) the distance between the perches where the bird waits until the follower has caught up; and (3) the height of such perches. On arrival at the nest, the bird perches close to it and gives a characteristic 'indication' call (this call, like the initial one, is documented in sonograms). The bird also hops among close perches, often circling the nest in between perching. Isack and Reyer remark that this behaviour of effectively reducing the 'step length' as the goal of the search is approached is another example of a pattern (often called area-restricted search) that is ubiquitous in nature, being found, for instance, in flies looking for sugar particles³, parasitoids searching for hosts to oviposit on or in³, or schistosome miracidia homing in on snails as intermediate hosts⁴.

Young honeyguide leaving the nest.

just completed a 3-year study in which they document the involvement of a third species in this association — humans.

Rock paintings in the Sahara, Zimbabwe and South Africa show that humans have collected honey in Africa for at least 20,000 years². Many anecdotes (the earliest dating back to the seventeenth century²) suggest that humans have cut into the honeyguide-ratel dance, using the bird to help guide them to honey sources. Sceptics have viewed these anecdotes as romantic myths, and the issue may soon become moot because in many areas honey is increasingly obtained from bee-keeping or is being replaced by these areas, the birds no longer guide.

Isack and Reyer's study¹ of the interactions between honeyguides and nomadic

Ardea/Peter Steyn

IMAGE
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How do the honeyguides find the nest to which they guide their ratel or human associates? One answer is that the birds know the location of colonies in advance, another is that the birds literally 'wing it' until the sight or sound of accidentally encountered bees gives a clue. The rather straight flight-paths recorded by Isack and Reyer argue indirectly for the former explanation. More directly, Isack and Reyer watched from blinds and saw several unaccompanied honeyguides visiting nests, apparently on tours of discovery and inspection (when the bees were relatively quiet, as on cool mornings, birds would actually fly into the entrance of a nest and peer into it).

Isack and Reyer's study is, of course, engaging in itself. But, as the authors observe, it also addresses a larger issue. Boran 'ecologists' knew all their conclusions, even though they had not buttoned them down with sonograms and Mann-Whitney two-tailed tests. To an even greater degree, in the tropical rainforests

of Africa, Asia and South America, about whose flora and fauna conventional science knows so little, a vast store of ecological knowledge resides among the diminishing groups of native people, who draw upon such knowledge for food-gathering, medicine and other aspects of daily life. Ethnoecology, ethnobotany and other such disciplines are in their infancy. They hold the promise of helping us answer important questions more quickly than will otherwise be possible. Yet these possibilities are disappearing, as the cultures of native people are being destroyed at an even faster rate than the forests and other places where they live. □

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1. Isack, H.A. & Reyer, H.U. *Science* **243**, 1343–1346 (1989).
2. Friedmann, H. *Bull. U.S. natn. Mus.* **208**, 1–15 (1955).
3. Hassell, M.P. *The Dynamics of Arthropod Predator-Prey Systems* (Princeton University Press, 1978).
4. Anderson, R.M. *Parasitology* **79**, 63–94 (1979).

OENOLOGY

In vino veritas

Robert W. Cahn

GRAPE juice is often 'enriched' with additional sugar, in the hope of enhancing the quality of the wine resulting after fermentation. The European Community (EC), in the shape of the Council of Ministers, occasionally debates whether or not to prohibit the practice, or to limit the amount and type of sugar that may be added, and new regulations will eventually be promulgated. However, such regulations would be pointless without a reliable method to detect the added sugar, after fermentation is complete. Such a method now exists¹, and the EC recently approved it as a standard Community procedure.

Jean Antoine de Chaptal (1756–1832)

was an agile French chemist who managed to keep his head while an eminent contemporary, Lavoisier, lost his to the post-revolutionary Terror. A landowner, Chaptal introduced the cultivation of sugar beet, the British blockade having prevented the normal importation of cane sugar. Having succeeded in this, he proposed that after summers with too little sun, grape juice be fortified with beet sugar so that it fermented as it should. Ever since, the process of fortifying wine in this way has been known among the French as *chaptalisation*. The *Supplement to the Oxford English Dictionary* defines chaptalization more broadly: "to convert or improve the must, in wine-making, by

neutralizing an excess of acid or adding sugar". In the EC's Council of Agriculture Ministers, the French and the Germans, dwellers in cool climes, are inclined to refer to it informally as enrichment; the British, more neutral in the matter, call it sugaring; and the Italians and Greeks, whose hot climates ensure that grapes are never short of sugar, complain of adulteration.

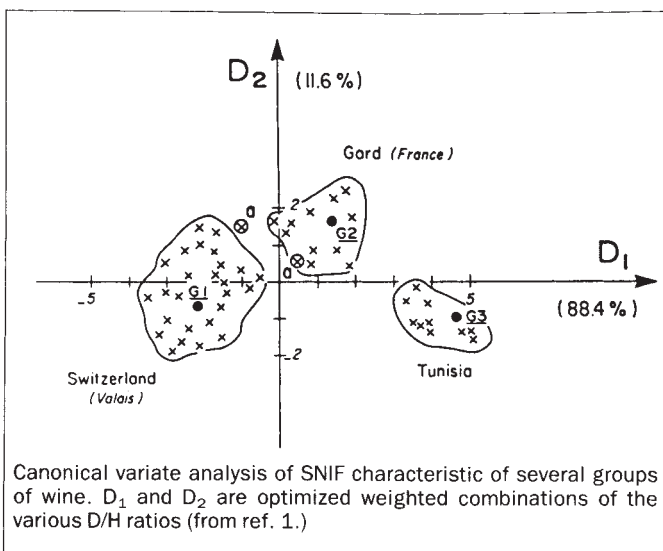
Whichever word is preferred, the

Commission has concluded that recent developments due to Gérard Martin's team at the University of Nantes will allow chaptalization to be identified *ex post facto*. Indeed, the French authorities, ever concerned to protect the reputation of French wines, some years ago offered a prize of 1 million francs for an effective method of detection, and Martin was the winner. Martin's most complete account of his technique was published last year¹.

Characterization of wines and distilled liquors has for a long time used one of two approaches: either sensory analysis, which is an impressive name for tasting and sniffing (see illustration), with a top-dressing of multivariate statistical analysis and psychology, or capillary gas chromatography, which identifies the numerous esters and other constituents which are responsible for flavour. These approaches are critically discussed in a recent book² and clearly have serious weaknesses — not least, the difficulty arising from the fact that the population of esters, and so on, is constantly changing as a wine matures. In the same book, Williams³ in particular examines the problems involved in tracking down a wine to its region of origin and in testing for chaptalization, and points to the special strength of the method introduced by Martin 'site-specific natural isotope fractionation' (SNIF).

The analysis of isotope ratios has, of course, become steadily more prevalent in geochemistry, environmental sciences and biochemistry; as practised in these disciplines, the normal tool is the mass spectrometer and isotope ratios refer to molecules or crystals as a whole. Attempts have been made to use the ¹³C/¹²C ratio to distinguish between sugars from different sources (grapes, cane, beet), as different plants follow different photosynthetic cycles. It is possible to distinguish with some confidence between wines fortified with cane sugar and unfortified ones³, but it seems that beet sugar poses an insoluble problem with this technique (though the adulteration with beet sugar of rum, made from cane sugar, is detectable).

The drawback of the traditional method of isotope analysis is that only the overall isotope ratio in a molecule can be measured. Martin's method overcomes this limitation by measuring the proportion between hydrogen and deuterium in a specific site within an organic molecule by nuclear magnetic resonance. This type of measurement can be used to 'fingerprint', in particular, alcohols fermented from different sugar precursors. Ethyl alcohol has two distinct carbon-bonded hydrogen sites, the methyl and methylene sites, and by measuring D/H ratios for these sites separately, as well as for the water constituent of wines, Martin and co-workers can pinpoint the botanical and (to a somewhat lesser degree of reliability) the geographical origin of the alcohol. ▶



Canonical variate analysis of SNIF characteristic of several groups of wine. D_1 and D_2 are optimized weighted combinations of the various D/H ratios (from ref. 1.)



Sentir le bouchon: olfactory sensory analysis will soon be displaced by SNIF-NMR¹. (From *Monseigneur le Vin*, Etablissements Nicolas, Paris, 1927.)

Martin and his wife, Maryvonne, first proposed¹ the SNIF-NMR method in 1981 and gave a much more detailed account² in 1986. In the meantime their son, Gilles, set up a company, Eurofins, in Nantes to exploit the patented technique, and the general assembly of the *Office International de la Vigne et du Vin*, a long-established intergovernmental body with 33 member states, in January 1987 after 4 years of tests approved SNIF-NMR as an officially recognized method of "displaying the chaptalization of wine" and the EC followed suit the following year.

In the SNIF-NMR method, the wine is distilled to obtain almost pure alcohol; this is necessary to enhance the precision of the NMR measurement, which depends on the alcohol concentration in the NMR sample. Recent research has established that, in general, results are more consistent if measurements are made only on the alcohol resulting from fermentation, not on the original must or grape-sugar solution. In fact, a recent (April 1988) resolution of the *Office International* has specified that only alcohol analyses be used for its purposes. The D/H ratios, which can generally be determined to a precision of 0.2 parts per million from the relative intensities of NMR lines, each identified with a distinct deuterated 'isotopomer', are calibrated against internationally standardized water samples³, against standard tetramethylurea and against a collection of sealed standardized alcohols fermented from different precursors, provided by the Community Bureau of References. Of course, the total weighted D/H ratio for an alcohol molecule taking into account all sites should match the D/H ratio obtained by mass spectrometry. According to Martin *et al.*¹, there is indeed quite good agreement but with a systematic deviation of a few parts per million, which has recently been traced to differences in the deuterium ratio of the mutually exchanging hydroxyl sites of

water and ethanol (G. J. Martin, personal communication).

Over several years, Martin's team analysed many certified wines from France and other countries represented by the *Office International*, seeking correlations of the D/H ratios (for the methyl and methylene sites in alcohol and for water) with factors such as grape variety, region of origin (and hence climate) and of course chaptalization. The statistical method used is canonical variate analysis, aided by a specialized computer diagnosis system, ISOLOG⁷. Different weighted combinations of experimental variables (here, D/H ratios) are systematically compared to find the one best correlated with a particular external factor. The figure shows the results of the test of the distinguishability of groups of wines from the south of France, Switzerland and Tunisia, and the statistical separation is clear enough. The method can often distinguish between wines made from different grape varieties. The D/H ratios seem to vary according to the sugar concentration in the various grapes, which itself is a function of geography, grape variety

and the weather preceding a vintage.

As things stand, Community regulations permit enrichment of grape must only with other (more concentrated) grape must, natural or rectified, except in those countries having a long tradition of adding beet sugar, which are free to follow their national rules. The Council of Ministers some time ago instructed the Commission to report to it on current enrichment practices; this report is due soon, and then the Council will have to try to reach agreement on future regulations for Europe as a whole. It undoubtedly is an extremely sensitive political issue. □

Robert W. Cahn is in the Department of Materials Science and Metallurgy, University of Cambridge, Pembroke Street, Cambridge CB2 3QZ, UK.

1. Martin, G. J. *et al.* *J. Agric. Food Chem.* **36**, 316 (1988).
2. Birch, G. G. & Lindley, M. G. eds *Alcoholic Beverages* (Elsevier, London, 1985).
3. Williams, A. A. in *Alcoholic Beverages* (eds Birch, G. G. & Lindley, M. G.) 129–144 (Elsevier, London, 1985).
4. Martin, G. J. & Martin, M. L. *Tetrahedron Lett.* **22**, 3525–3528 (1981).
5. Martin, G. J. *et al.* *J. Am. chem. Soc.* **108**, 5116 (1986).
6. Gonfiantini, R. *Nature* **271**, 534–536 (1978).
7. Martin, G. G., Pelissolo, F. J. C. & Martin, G. J. *Computer Enhanced Spectroscopy* **3**, 147–152 (1986).

ELECTRON TRANSFER

Energizing protons in membranes

R. J. P. Williams

In 1961, two proposals were made as to the way in which electron-transfer reactions of the cytochrome chain — the chain used in the oxidation of NADH by molecular dioxygen — could be connected to ATP formation without the intervention of chemical intermediates^{1,2}. Both mechanisms invoked the transduction of the energy of the oxidation/reduction reaction to a proton gradient before the gradient generates ATP. The two mechanisms, sometimes termed the delocalized (Mitchell) hypothesis and the localized (Williams) hypothesis, are very different. In the first, protons generated by oxidation appear only in aqueous phases. Even ATP is generated by an electric field acting on the ATP synthetase and not by proton flow. In the second, protons move in proteins within matrices and aqueous phase equilibrations are ignored in the development of proton gradients, in proton diffusion and in the ATP-synthesis step. To distinguish between these mechanistic possibilities, long series of experiments have been carried out on separate parts of the cytochrome chain. The most decisive attack on the last stages of the electron-transfer reactions, those of cytochrome oxidase, has been carried out by Wikström and his collaborators³. Wikström reports his latest data on page 775 of this issue⁴.

Wikström and colleagues first showed that apart from the protons which react

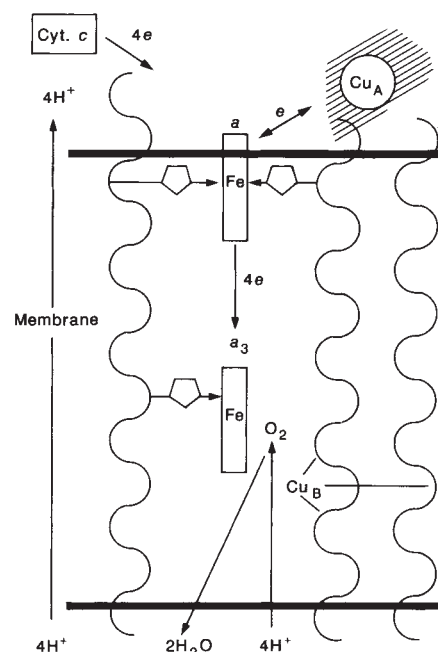


FIG. 1 Cytochrome oxidase (refs 5,6). The reaction centre binds dioxygen which is converted to water by the removal of protons from one side of the membrane (bottom). All the dioxygen reactions take place at the a_3 - Cu_B site while electrons come into the site from cytochrome *c* via haem *a* and Cu_A . The protein has several (putative) transmembrane helices and some, not all shown, are involved in proton pumping. One H^+ for each electron is used stoichiometrically.

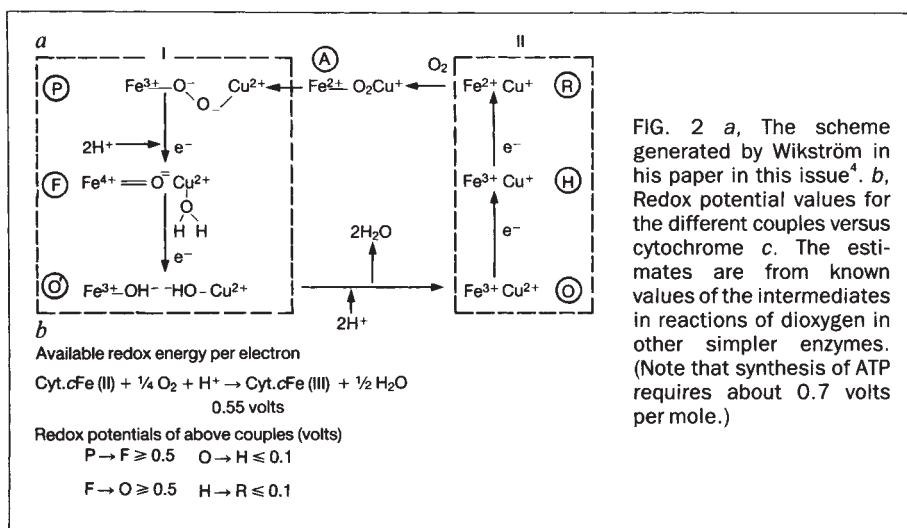


FIG. 2 *a*, The scheme generated by Wikström in his paper in this issue⁴. *b*, Redox potential values for the different couples versus cytochrome *c*. The estimates are from known values of the intermediates in reactions of dioxygen in other simpler enzymes. (Note that synthesis of ATP requires about 0.7 volts per mole.)

directly with dioxygen and which must diffuse through the protein matrix to the reaction centre in the membrane, additional (one H^+ per electron) protons are in fact energized by the reactions of molecular dioxygen in the membrane, and that these protons are pumped (in proton channels) across the membrane by gated energized diffusion³. The nature of the gating is as yet unknown. In an effort to elucidate the reaction path, Wikström now considers more precisely the coupling steps between the reduction of dioxygen by electrons and this proton pumping⁴. He concludes that proton pumping, which, note again, is separate from the protonation steps of dioxygen itself, occurs in association with the reaction steps involving only the first and second electrons of the four required to convert O_2 to $2\text{H}_2\text{O}$. I shall now consider some consequential mechanistic features.

In a protein such as cytochrome oxidase, consisting in all probability of membrane helices^{5,6}, cooperativity should occur via conformational changes on O_2 uptake or electron transfer as in haemoglobin and haemocyanin. In fact the a_3Cu_b centre of the cytochrome oxidase can be thought of as half haemoglobin and half haemocyanin (Fig. 1). Now the reactions of these proteins and of oxidases such as cytochrome P_{450} and even of cytochrome *c* with ligands are known to occur from one side of the proteins only via groove-opening reactions. It is hardly surprising, then, that the reactions of cytochrome oxidase, $\text{O}_2 + 4e + 4\text{H}^+ \rightarrow 2\text{H}_2\text{O}$, occur through the oxidase from only one side of the membrane in which the protein is lodged. The apparent surprise is that the additional proton pumping in a different, helical channel is driven by the first two steps of reduction only.

Let us consider the steps. Each of the steps $\text{R} \rightarrow \text{P}$, $\text{P} \rightarrow \text{F}$, $\text{F} \rightarrow \text{O}$, $\text{O} \rightarrow \text{H}$ and $\text{H} \rightarrow \text{R}$ are expected to be linked to conformational changes (Fig. 2 and see Fig. 1). The step $\text{R} \rightarrow \text{P}$ is not reversible. Whatever the series of conformational switches, the

later steps return the protein to its initial state in a cyclic, irreversible manner and so cannot be the forward strokes of a proton pump. Again, the redox energy of dioxygen relative to cytochrome *c* is largely lost going from $\text{P} \rightarrow \text{O}$ because the last two one-electron potentials of the oxidase are not far from those of cytochrome *c* itself (Fig. 2). It is good sense that the cyclic functioning of a pump has highly energy-using initial steps and later relaxation steps driven by little energy loss.

How does this pump compare with other ion pumps, such as ATP-synthetase, Na^+/K^+ ATPases and Ca^{2+} ATPases, and bacteriorhodopsin? In all these cases it has been supposed that cyclic helix/helix motions of proteins are part of the mechanical device in the pump. Moreover, it seems that the sites of energy input (here of ATP to the ion movements) are far in space from the gated channels, which are frequently considered to be constructed from anionic sites on helices within the membrane. The parallels with cytochrome oxidase are fairly close. Moreover in several cases where intermediates, such as phosphorylation of proteins, have been seen, the last steps, now loss of inorganic phosphate, act not in the pumping stroke but in relaxation, and release little energy.

It seems that several pumped movements of ions and possibly molecules in proteins in membranes have common features — helical protein structures, cycling conformation changes and readily distinguishable forward pumping and return steps where energy commitment is largely in the first steps. □

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- Williams, R.J.P. *J. theor. Biol.* **1**, 1–13 (1961).
- Mitchell, P. *Nature* **191**, 144–148 (1961).
- Wikström, M. & Saraste, M. in *Bioenergetics* (ed. Ernster, L.) Ch. 3 (Elsevier, Amsterdam, 1984).
- Wikström, M. *Nature* **338**, 775–778 (1989).
- Holm, L., Saraste, M. & Wikström, M. *EMBO J.* **6**, 2819–2823 (1987).
- Williams, R.J.P. *FEBS Lett.* **226**, 1 (1987).

Blow the fuse!

LABORATORIES all round the world are trying to replicate the claimed electrolytic cold nuclear fusion from heavy water. Some may regard this as premature when the one crucial control experiment, the equivalent electrolysis of light water, has not yet been tried. But with his usual optimism, Daedalus has set DREADCO's electrochemists to forcing deuterium into palladium electrodes, and is determined to interpret any excess heat production as nuclear fusion, rather than some banal chemical or lattice effect.

Palladium has to take up a lot of deuterium before any heat is disengaged at all; but thereafter things seem to accelerate rapidly. This makes obvious sense: each site in the palladium lattice will take up one deuterium atom until all the sites are full. Further deuterium atoms will then enter already-occupied sites, and these close-packed pairs stand a chance of fusing together by quantum tunnelling. The energy released can, apparently, even melt the palladium.

But Daedalus goes further. He points out that as two nuclei approach each other, the chance of successful quantum tunnelling rises dramatically. So a sphere of deuterium-saturated palladium would make an ideal implosion-style hydrogen bomb. At atmospheric pressure it would release a steady heat flux and an inconvenient but containable neutron flux. Surround it by shaped charges of high explosive and crush it suddenly to half its volume, and the result should be a splendid combined fusion explosion and neutron emission. Despite the deadly momentary neutron flux, this elegant bomb would release almost no enduring fallout: a valuable military advantage. Lacking a critical mass, it could also be made in usefully small sizes.

Daedalus is rather diffident about contributing to weapons research. In shifty self-defence he claims that nuclear physics is supported so lavishly by national governments only because it once produced a bomb. To keep the funds coming in, the subject really has to repeat the trick from time to time. In any case a pure, hygienic, noble-metal fusion weapon is a great ecological improvement on nasty poisonous and radioactive fission weapons, and should be supported enthusiastically by the increasingly militant 'green' political parties. But in more peaceful vein, Daedalus is also devising a fusion-powered watch. Each time its oscillating palladium hair-spring flexes, deuterium fusion accelerates on its compressed face, and slows down on its tensioned face. The differential thermal expansion unwinds the spring; the inertia of the balance wheel drives it the other way, generating the opposite thermal gradient, and so on. A tiny charge of deuterium should run it for centuries. David Jones

Cold fusion: what's going on?

SIR—A significant point which is not widely known, and may therefore be overlooked in neutron measurements of cold fusion rates, is the possibility of contamination by cosmic-ray-generated neutrons; these should be taken into account in the design and interpretation of experiments.

The cosmic-ray-induced neutron background arises primarily from extra-solar protons with energies above a few GeV, which can penetrate the Earth's atmosphere and the Sun's and Earth's magnetic fields¹. Primaries and secondaries reaching the surface include neutrons and other energetic particles which produce neutrons in the atmosphere and the first few metres of the surface by spallation reactions. While the spectrum contains neutrons up to energies comparable to incident particle energies, a major component is due to evaporation of neutrons from struck nuclei; at birth these have energies in the range 1–3 MeV, and appear as a knee or shoulder on an otherwise continuous energy distribution. There is also a substantial component consisting of 'thermal' neutrons, which have slowed down in the environment to a poorly equilibrated thermal distribution below energies of about 0.1 eV as well as 'epithermal' neutrons whose distribution is roughly inversely proportional to the energy in the range 1 eV to 1 MeV. At energies above a few MeV, the spectrum tails off rapidly; this cascade component contains about 10% of the total. The flux of each of the low-energy components is of the order of 10^{-2} neutrons $\text{cm}^{-2} \text{s}^{-1}$ at sea level and middle latitudes.

These figures vary according to altitude (about twice as great at 1,500 m elevation), and from time to time, mostly because of variations in atmospheric density and solar and geomagnetic field intensity. The e-folding thickness in the atmosphere is about 150 g cm^{-2} , so that, for example, barometric pressure variations of $\pm 13 \text{ mm}$ of mercury cause about $\pm 10\%$ flux variations. Sometimes, when dealing with such a general source of neutrons, material intended as shielding, and even detector material itself, can act as a source, so that some care in this respect is called for in the measurements.

As it happens, the counting rates due to cosmic-ray-induced neutrons are of the same order of magnitude as the counting rates observed in the neutron and secondary radiation detectors in many of the measurements being made. And in detectors that disperse the spectrum, the evaporation peak in the energy distribution due to cosmic-ray-induced neutrons is at nearly the same energy as that expected from deuterium-deuteron fusion, 2.45 MeV. These observations, coupled with the (admittedly weak, $\pm 10\%$)

temporal (hourly, daily) variation of the cosmic-ray-induced neutron fluxes require that this background be carefully accounted for.

Comparable neutron fluxes can be generated by accelerators, isotope sources and nuclear reactors, even at considerable distances; these contaminants of neutron measurements must also be reckoned with. Obvious means for suppressing these backgrounds are time-gating of the source, monitoring spurious sources with a second detector operated simultaneously with the detector(s) near the source under investigation, or going underground— 350 g cm^{-2} (two or three metres of earth or concrete on all sides) should reduce the cosmic-ray neutrons by a factor of about 10.

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1. Hayakawa, S. *Cosmic Ray Physics* (Wiley, New York, 1969).

Dr Carpenter, a referee of the paper by Jones *et al.* on page 737, provided this comment at our invitation.

The following are extracts from the substantial numbers of letters from readers offering explanations of the two series of cold fusion experiments which have been generally reported.

Editor, *Nature*.

SIR—From the newspaper accounts, the very small flux of neutrons generated during the experiment of Fleischmann and Pons is being taken as proof that their conclusion is not valid, and that nuclear reactions between deuterons do not occur under the conditions they describe.

But when the kinetic energy is as small as in their experiment, the neutron and proton components of the deuteron do not behave in the same way, because the nucleus of the target atom repels the proton but not the neutron. Thus, the neutron can be captured by the target nucleus while the proton, which remains outside the Coulomb barrier, will fly off.

This process, first recognized by Oppenheimer and Phillips¹ in 1935 leads to a pure (*d, p*) reaction and has a relatively high probability of occurrence, certainly much greater than that of the (*d, n*) reaction².

It follows that if the experiments described really brought the deuterium nuclei close enough together to interact, one should expect no neutron emission and a reaction rate much higher than that evaluated on the basis of the high-energy

model.

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SIR—The only recognized mechanism for nuclear fusion at ambient temperature is that induced by negative muon binding of precursor hydrogen isotope molecules as first described by Frank¹ with estimates of fusion rates by Sakharov² and experimental observations by Alvarez³ some 30 years ago. It is now known that more than 100 fusion events may be induced by a single muon during its lifetime of 2.2×10^{-6} seconds in a mixture of liquid deuterium and tritium⁴. If this number could be increased by a factor of 1,000, a break-even fusion reactor could be the result⁵.

It is tempting to interpret the recent claims in terms of this process. It should be remembered that more than 70 per cent of the cosmic-ray flux at the Earth's surface consists of positive and negative muons. There are about $200 \text{ m}^{-2} \text{s}^{-1}$ with a stopping rate in an absorber of some $2 \times 10^{-5} \text{ g}^{-1} \text{s}^{-1}$. The flux may be twice as great at the altitude of Salt Lake City⁶, which would be equivalent to more than two muons a minute in an absorbing volume of about a litre.

The rate at which fusion events could occur is limited by the rate of formation of muon-bound molecules, which is itself a sensitive function of parameters on the atomic scale, whence its dependence on resonance effects⁷ and temperature⁸. Once a muon is captured, the resulting muonic molecule is two orders of magnitude smaller than the typical lattice spacing in solids, so that free diffusion may be expected.

The fact remains that muon-induced fusion has not yet been reported in metallic compounds of hydrogen isotopes, and indeed has been considered unlikely because of the preferential capture of muons by the heavier metal nuclei. On the hypothesis that this loss mechanism is suppressed by a resonance or band structure effect in deuterium-loaded palladium, it is possible to estimate the turnover number required to explain the effects which have been reported.

Jones *et al.*⁹ observe a neutron count-rate of $4 \times 10^{-3} \text{ s}^{-1}$ with a neutron detection efficiency of about 1% in a volume of 160 ml. If the neutrons observed are products of the reaction, in a muon-deuterium molecule, of two deuterons to yield ³He and a 2.45-MeV neutron, one should also allow for the equally probable reaction yielding ³H and a proton, which would not have been detected by the neutron monitor. This implies five fusion events per second per litre. The required turnover number is then of the order of 100, comparable with that already known for the case of a mixture of liquid isotopes, but significantly greater than that in pure

1. Oppenheimer, J. R. & Phillips, M. *Phys. Rev.* **48**, 500 (1935).

2. Morrison, P. *Experimental Physics* Vol. 2 (ed. Segrè, E.) (Wiley, New York, 1953).

deuterium.

The consequence is that fusion induced by cosmic-ray muons cannot be excluded as an explanation of the reports of radiation effects in palladium loaded electrochemically with deuterium, although the estimates of the rate of muon-induced fusion is much less than that required by the thermal observations of Fleischmann and Pons¹⁰.

These developments emphasize the need for experimental data on the effects of negative muons in solids, especially metal deuterides and tritides, which are at present lacking in the open literature. Arrangements are in hand to investigate the reported palladium-deuterium effects with a muon source of greater flux than that of the natural cosmic ray radiation.

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1. Frank, F.C. *Nature* **160**, 525 (1947).
2. Sakharov, A.D. *Report of the P.N. Lebedev Inst.*, Moscow (1948).
3. Alvarez, L.W. *Nobel Lecture* (1948).
4. Breunlich, W.H. *Muon Catalyzed Fusion* **1**, 29 (1987).
5. Gajewski, R. & Jones, S.E. *Muon Catalyzed Fusion* **2**, 93 (1988).
6. O'Sullivan, C.T., PhD Thesis (Catholic Univ. Amer. Press Washington DC 1969).
7. Dzhelepov, V.P. *Muon Catalyzed Fusion* **2**, 9 (1988).
8. Balin, D.V. *et al.* *Muon Catalyzed Fusion* **2**, 241 (1988).
9. Jones, S.E. *et al.* Univ. Arizona preprint No. AZPH-TH/89.
10. Fleischmann, M. & Pons, S.J. *electroanal. Chem.* **261**, 301-308 (1989).

SIR—In my theoretical investigations of the electronic structure of the H_2^+ molecule (*Phys. Lett.* **123**, 170; 1987), I have found that the two nuclei and the electron can form a collapsing quasi-molecule — a compound system whose dimensions decrease with time to zero. (The extreme case is when the electron is at the centre-point between the two nuclei.) In general, in collapsing molecules like these, the repulsive Coulomb interaction of the nuclei and the gas-kinetic pressure of the electron are less than the attractive Coulomb forces between the two nuclei and the electron. The closest approach of the two nuclei depends on the initial state of the electron, its binding energy and the mean value of the kinetic energy in particular.

The probability of the tunnelling effect is therefore identical with the probability of formation of a collapsing quasi-molecule. Thus it is clear that the electrons present in the matter are responsible for the Coulomb-barrier tunnelling, and that the process which has been observed depends on quasi-molecular systems.

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SIR—Reports of the experiments by Fleischmann and Pons contain a paradox

— that, if fusion reactions do occur in them, either too much energy is liberated or too few neutrons are detected. I wish to suggest a possible explanation.

I start from the hypothesis that the palladium contains regions where the density of deuterons is sufficiently great for d-d fusion to occur by one of the reactions leading either to 3H and a proton or to 3He and a neutron. The product particles will be produced within a region where the density of other particles is very great. The mean free paths of the particles will then be very small, and it appears reasonable to assume that even though these high-density regions will be geometrically small, they will be so optically large that even the most penetrating particles, such as neutrons, will remain trapped inside them. In this situation, the particles produced by fusion reactions will undergo multiple scattering collisions until a new reaction occurs.

Several such reactions are possible, including fusion reactions of deuterons with 3H and 3He (yielding 4He and a neutron or proton respectively) and the radiative capture of protons or neutrons by deuterons. These reactions are exothermic, releasing large amounts of energy.

It is crucial that these processes can also form multiplicative chains, especially if the γ -ray photons released by radiative capture reactions yield further energetic neutrons and protons by the photodisintegration of deuterons. The reaction chains will come to an end only when reactive particles escape from high-density regions to those where the density is insufficient to sustain them.

The main products of these reaction chains will be α -particles, but the reactive particles such as neutrons and 3H will only infrequently be released to the environment.

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Pulsar formation

SIR—Lindley¹ states that the report^{2,3} of a half-millisecond pulsar in the remnant of supernova 1987A “has surprised everybody and could, if confirmed, stand theory on its head”. He has apparently overlooked several papers (refs 4 and 5, for example) suggesting that pulsars may form directly as rapidly rotating neutron stars “in original spin” with weak magnetic fields, without the need for “resurrection” after birth as a slow rotator followed by subsequent decay of the magnetic field and spin-up by accretion of matter from a companion star⁶.

Even before the discovery of millisecond pulsars, we discussed⁷ the question of why collapsed stars rotate so slowly,

and pointed out that if there was significant mass ejection during the formation of a neutron star, and if it had a strong magnetic field, it was likely to be born spinning slowly. We argued, furthermore, that if the progenitor giant core of the neutron star has a strong magnetic field, it is likely to have been rotating relatively slowly, as the magnetic field would have enhanced transfer of angular momentum from it to the envelope during earlier evolutionary phases. We concluded that if weak-field neutron stars could form at all, they would be born spinning fast. If the optical emission from the half-millisecond pulsar arises from incoherent synchrotron radiation at the light cylinder, and scaling by the optical luminosity and magnetic field of the Crab pulsar, it follows that the half-millisecond pulsar indeed has a weak magnetic field of $B \sim 10^8$ gauss.

With the discovery of six millisecond pulsars (with periods ≤ 12 ms), of which four are in binary star systems, several authors concluded that ‘resurrection’ was required to account for their observed properties. A key requirement for the ‘resurrection’ model is that the magnetic field of neutron stars must decay on a timescale of a few million years. But arguments against significant neutron-star magnetic-field decay have been proposed (for example, ref. 8). Furthermore, calculations by Sang and Chanmugam⁹ showed that there are serious difficulties with all models so far proposed for field decay. In addition, there is no satisfactory detailed model that explains how single millisecond pulsars can be formed from binary star systems. Even in the case of PSR1957 + 20, where there is evidence of matter evaporating from the companion, the spin-down rate indicates insufficient energy loss from the pulsar to evaporate the entire companion star. When combined with all the difficulties of the ‘resurrection’ model, the discovery of the half-millisecond pulsar seems to provide substantial further support for the view that at least some millisecond pulsars can be born “in original spin”.

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1. Lindley, D. *Nature* **337**, 595 (1989).
2. Middleditch, J. *et al.* *IAU Circ.* No. 4735 (1989).
3. Kristian, J. *et al.* *Nature* **337**, 234–236 (1989).
4. Brecher, K. & Chanmugam, G. *Nature* **302**, 124–125 (1983).
5. Arons, J. *Nature* **302**, 301–305 (1983).
6. Ruderman, M. & Shaham, J. *Comments Astrophys.* **10**, 15–22 (1983).
7. Brecher, K. & Chanmugam, G. *Astrophys. J.* **221**, 969–972 (1978).
8. Kundt, W. *Comments Astrophys.* **12**, 113–121 (1988).
9. Sang, Y. & Chanmugam, G. *Astrophys. J.* **323**, L61–L64 (1987).

On the bright side

Steven Rose

Biology and Freedom: An Essay on the Implications of Human Ethology. By S.A. Barnett. Cambridge University Press: 1989. Pp. 376. £32.50, \$39.50.

I'M LEERY about books with the words 'biology and . . .' in the title — they tend to contain yet more angels-on-a-pin discussions by philosophers about the ethical implications of science-fiction genetics, or sociologically naive speculations by biologists about the inevitability of war, the nuclear family and capitalism. *Biology and Freedom* comes as a refreshing contrast. Professor Barnett is a distinguished ethologist of the school which pre-dates the present-day obsessions with optimal foraging strategies, cost-benefit analyses and genetic fitness calculations, and his previous books include an authoritative study on the social behaviour of the rat. As anyone who has had the good fortune to hear one of his forceful contributions to that great Australian radio institution, *The Science Show*, will know, he does not suffer fools lightly. But his new book goes beyond the mere debunking of certain currently popular biologicistic follies, to offer instead a vision of a humanity set free by our very biological constitution.

Barnett's intentions are perfectly revealed in the epigram from Shaw with which the book opens: "Is human nature incurably depraved? If it is, reading this book will be a waste of time". The first two-thirds of the subsequent text is devoted to a critique of those claims for such incurable depravity that have found their way into three decades of biological textbooks and Sunday-newspaper popularizations. The final third offers us the author's alternative vision. The targets for his criticism are designated respectively *Homo pugnax* (the claim that human beings are innately violent and aggressive); *Homo egoisticus* (the claim that we are innately selfish); and *Homo operans* (the claim that we are innately greedy).

Behind the first title shelter the pre-sociobiological generation of pop-ethologists such as Lorenz and Ardrey (in contradistinction to the recent obituaries, Barnett has no hesitation in referring to Lorenz's youthful Nazi enthusiasms and more recent New Right leanings). The claim of selfishness, of course, refers to the DNA-centric view of the universe offered by sociobiology's 'modern synthesis', and provides Barnett with the context for a synoptic account of neo-darwinian evolutionary theory. Greediness turns out to mask the skinnerian vision of human beings as driven exclusively by contingencies of reinforcement, of reward and punishment, as epitomized in the utopian/dystopian

vision of *Walden Two*. There have been numerous critiques of these positions, of course. E.O. Wilson himself described the writings of Ardrey and Lorenz as "mere advocacy". The sociobiologists have been criticized on scientific, ideological and philosophical grounds (this last especially by Philip Kitcher in his book *Vaulting Ambition*, which oddly does not appear in Barnett's extensive bibliography). Chomsky's polemic with Skinner is well known.

Barnett, bringing to these debates a combination of deep familiarity with the ethological and anthropological literature and a gentle wit, has little difficulty in exposing the shallowness of each of the three visions of humanity. He leaves unaddressed, however, the question of just why these views have achieved the popular resonance, and their authors the fame, that they undoubtedly have. Just why should such poorly predicated and evidentially weak ideas continue to be taken seriously? For some, the answer lies in the part they play in helping set political agendas; Barnett allows his readers to pose and answer such questions themselves. This reticence may be appropriate in a book which is evidently designed, as this one is, to serve as a teaching text in courses in the human sciences as well as for a wider audience.

It is to the last third of the book that one must turn to move beyond critique and to

arrive at the author's own vision of human nature, the vision which informs the book's title. Reductionism and determinism fail as methods of understanding the human condition because in considerable measure our future is undetermined — we make our own history, though in circumstances not of our own choosing. Human beings are playful animals, curious animals, animals who make music, animals for whom work is not necessarily labour but can be liberating, unalienating. And human beings are also animals who teach. The reciprocal pleasures of teaching and learning, of the relationships between adults and children, are beautifully conveyed, both in prose and in many of the photographs which enliven the book.

Barnett's message is unfashionably optimistic. Certainly science and technology are associated with great horrors — and with the peculiarly distorted visions which the first portion of the book is concerned to dispose of. Equally certainly, human perfectibility is not an option. When television screens are full of images of war and starvation, when fanatical fundamentalists can condemn to death a writer who dares to laugh, it is hard to hold on to a utopian perspective. But Barnett remains unshakably a progressive. We do our present age an injustice, he argues, if we cling to ideas of human beings as greedy, violent and selfish, and fail to realize not only how far we have to go, but also how far we have already travelled towards a free, egalitarian and democratic society, a society which it is in accord with our biological natures to create. □

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Threatening gesture — the male three-spined stickleback *Gasterosteus aculeatus* in a posture that intimidates other males of the same species, here seen intimidating its own reflection. The behaviour is innate, and depends on both internal motivation and external sensory factors. The picture is taken from a reprint of *The Study of Instinct* by Nikolaas Tinbergen, who died last year (see S.A. Barnett's obituary in *Nature* **337**, 509; 1989). The book contains the original text written in 1949, along with an introduction added in 1969 and a brief 1988 preface. Publisher is Clarendon, price is £12.95.

Dirty deeds

John H. Simpson

Pollution of the North Sea: An Assessment. Edited by W. Salomons, B.L. Bayne, E.K. Duursma and U. Förstner. Springer-Verlag: 1988. Pp.687. DM 198, £65.

LAST summer the seals of the North Sea died in their thousands as an epidemic of canine distemper swept through a population whose numbers had previously been increasing. The episode, accompanied by intense media attention (it was the silly season), served to alert us all to pollution in the North Sea.

Ironically, it now seems unlikely that there was any direct connection between the seal deaths and toxic pollutants. But the plight of such appealing creatures (jelly fish would not have been the same) served as a powerful focus for public fear that something is seriously wrong in the ecosystem of Europe's shelf seas, and that remedial action is needed to avoid catastrophe. Marine scientists are now rightly being pressed to state what exactly are the pressures on the North Sea and to what extent they constitute an urgent threat. In *Pollution of the North Sea*, a wide-ranging and timely survey, the editors have attempted to assemble the best available

Disastrous legacy — the explosion on Piper Alpha in July 1988 killed over 150 workers and may have released toxic compounds into the North Sea.

scientific answers to these questions, and to express what they identify as the remarkable consensus amongst the scientists involved as to the vulnerability of the North Sea and its finite capacity to assimilate waste. That does not mean that there is no disagreement about the details; with so little knowledge in some areas there is still plenty of room for different interpretations and hypotheses about mechanisms. But there is a determination manifested in this volume to establish a scientific assessment of the problem undistorted by national political pressures.

With 40 separate papers by more than 70 authors, the book covers most aspects of the subject. The first section reviews the basic physics, chemistry and biology of the North Sea, giving due weight to the importance of suspended particulates — these absorb many of the pollutants, carrying them into the sediment where they are exposed to diagenetic processes before possible resuspension brings them back into the water column. Also here is discussion of the role of the surrounding sediment traps (salt marshes, estuaries and fjords) which act as sinks for pollutants, and of the natural variability of the system which so much complicates the problem of detecting the incipient consequences of pollution.

Section 2 provides a useful compendium of facts and figures on the main inputs of pollutants into the North Sea including, for example, best estimates of how much cadmium, mercury and lead (the latter mainly from the atmosphere) are entering each year. Some of the inputs, notably of organic pollutants, are hard to quantify. It is difficult to determine the concentrations of such a large number of compounds, and there are uncertainties surrounding single catas-

trophic events such as the Piper Alpha disaster, which may have resulted in the release of several tons of polychlorinated biphenyls, and the recent loss of a large quantity of Lindane in the Channel. The inputs of nutrients, on the other hand, are certainly increasing dramatically; the amounts of nitrate and phosphate coming from the Rhine double about every 15 years, and there is evidence of consequent eutrophication in the German Bight.

Pollutant effects in particular areas and on particular sections of the biota are considered in more detail in the two final sections. We learn, for example, about the specific problems in marginal areas such as the Wadden Sea, where pollution pressures are most intense and where we might look for early warning of the consequences. The Wadden Sea offers some of the few examples (for instance decline of the harbour seal, and changes in the populations of spoonbill and sandwich tern) where we have good evidence of the impact of pollutants on particular species.

Given so many contributors, the scientific quality of this large volume is inevitably somewhat variable. Although the choice of material is, on the whole, balanced, there has been no attempt at synthesis in the form of overall and sectional synopses. This, I fear, will make the book harder to use and less accessible to the non-specialist than it might have been. Nonetheless, *Pollution of the North Sea* is a unique and valuable compilation of material. It will serve as a vital source of reference for those involved in the science and management of the North Sea, and in the presentation of the attendant issues to a wider public. □

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The opposite of knowledge

John S. Rigden

The Privilege of Being a Physicist. By Victor F. Weisskopf. W.H. Freeman: 1989. Pp. 235. \$17.95, £12.95.

It is appropriate that the most prominent word in the title of Victor Weisskopf's book is *Privilege*. Through talent and hard work, Weisskopf has gained an international reputation and has attracted many honours; yet he is that rare kind of man who regards it all as a privilege.

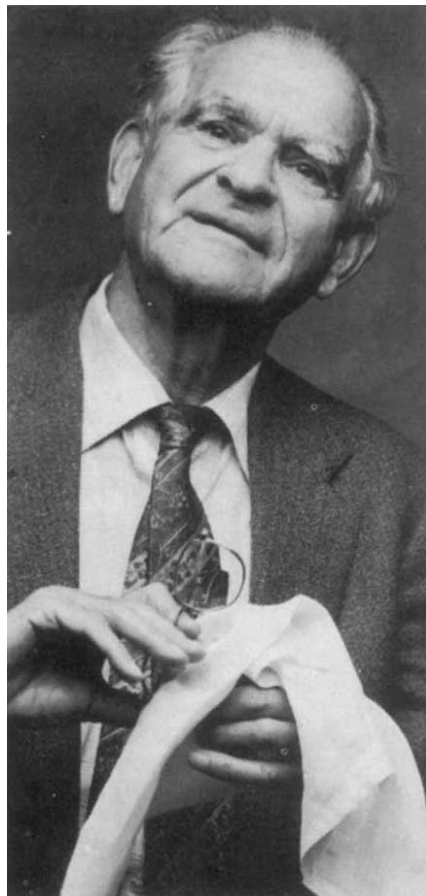
For readers familiar with two of Weisskopf's earlier books, *Knowledge and Wonder* (Doubleday, 1962) and *Physics in the Twentieth Century* (MIT Press, 1972), the appearance of this new work will be a happy event. It brings together 16 essays: two were written specifically for the book, the others have mostly appeared over the past ten years (although four of them have been expanded for the present collection). The subjects covered are some of the principal ideas of twentieth-century physics, two of the physicists who participated in the creation of those ideas, and the relationship of science to culture and to society.

The theory of quantum mechanics was created during the years 1925–1927. For those who followed events, it was a time of intellectual exhilaration and purgation. Conundrums that had plagued physicists during the early years of the twentieth century were suddenly resolved — provided they were willing to accept nature on entirely new terms and think in entirely new ways. To engage one's mind in thinking about such a conceptually rich subject, and to do so in the fundamental ways that were required to create the formalism of quantum mechanics, is an experience that alerts the mind to the broad place of physics in human activities.

With just a touch of sadness, Weisskopf has acknowledged that he came to physics "three years too late" — too late, that is, to be an active participant in the creation of quantum mechanics (*Physics in the Twentieth Century*, p.4). Starting in 1929, however, he made many seminal contributions through the application of quantum mechanics to our understanding of matter. Moreover, he has been one of those theoretical physicists who has thought deeply about the ideas of quantum mechanics and about the interpretation of this incredible theory. In addition, he knew personally men such as Bohr, Heisenberg and Pauli who were the architects of quantum mechanics and he bears their influences. This is the background that Weisskopf brings to his writing.

Although they were written for the

general reader, scientists will also find the essays edifying. Weisskopf does not present physics as an activity isolated from values and emotions. Quite the contrary. He shows us how physics and the humanities arise from the same universal values and complement each other. Teachers at all levels would be well advised to read and ponder the contents of the book. Too often science instruction takes the form of transmitting bits of knowledge that are isolated from any scientific, cultural or human context. But such bits of knowledge provide students with no under-



Weisskopf — a rare kind of man.

standing of science. Weisskopf goes further: in the chapter "Teaching Science", he asserts that "science is the opposite of knowledge" and portrays science as the product of curiosity and questioning which can lead to a "fuller, more meaningful life".

Weisskopf is at his best when he writes about the ideas of physics. In "What is Quantum Mechanics?", written specifically for this collection, we learn how "the primal shapes of nature" bring stability to atoms and how the uncertainty inherent in nature really brings the certainty that renders all atoms of a given element identical. This essay, along with "What is an Elementary Particle?", "Contemporary Frontiers in Physics" and "The Origin of the Universe", reveals not only the depth of Weisskopf's understanding, but also demonstrates his expository skill. For

their clarity and insight, these essays are outstanding.

Wolfgang Pauli and Werner Heisenberg are each the subject of Weisskopf's attention. Pauli is typically the subject of many anecdotes; Weisskopf adds to them and, in the process, brings insights to Pauli's intriguing personality. In the case of Heisenberg, Weisskopf's admiration of the man prompts an explanation of his wartime activities; this account is based largely on *Inner Exile* (Birkhäuser, 1984), the recent book by Heisenberg's widow, and in comparative terms it is somewhat more generous than the findings of contemporary historians such as Alan D. Beyerchen (*Scientists Under Hitler*; Yale University Press, 1977).

Implicitly, Weisskopf extends the same generosity to American physicists. If quantum physics can be said to have had a determining influence in Weisskopf's life, so can his experiences at Los Alamos. In 1943, Hans Bethe persuaded Weisskopf to join the team at Los Alamos and work on the development of the atomic bomb. Weisskopf became one of the prime movers in the Manhattan Project. When the war ended, however, he refused to participate in the further development of nuclear weapons; rather, he helped to form the Federation of Atomic Scientists, a group whose purpose was to alert the public to the dangers of nuclear war. In making this decision, Weisskopf differed with many of his fellow physicists who on the one hand called for an end to the arms race and on the other continued to develop successive generations of nuclear weapons. As the history of the post-war period is now being written, it is apparent that one of the primary driving forces of the arms race has been the ability and willingness of physicists to realize technical possibilities which have inevitably proved irresistible to political and military leaders. In this regard, Weisskopf might have expanded his concept of the privilege of being a physicist to include more directly and more insistently the idea of responsibility. As for himself, Weisskopf's responsibility has been fulfilled: in the book he continues to call for an end to the madness of nuclear weapons.

The essays were written over a period of years, so there is some repetition; I suggest that each of them should be read individually (rather than consecutively). In this fashion, they will reinforce each other while each is savoured. □

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• Part 2 of Solly Zuckerman's memoirs, *Monkeys, Men, and Missiles: An Autobiography 1946–1988*, has been published in the United States by W. W. Norton. Price is \$22.50. The British edition was reviewed by William Cooper in *Nature* 336, 285 (1988).

Pleasing all of the people

C. J. Gilmore

General Chemistry. By P. W. Atkins. W. H. Freeman: 1989. Pp.989. Hbk \$49.95, £32.95; pbk \$39.95, £16.95.

WRITERS of textbooks on general chemistry have a problem that their colleagues in subjects such as biochemistry, cell biology and computer science (to name but three examples) do not have. Chemistry is an old subject, many of its foundations being laid in the nineteenth and early twentieth centuries. Thermodynamics, for example, is a product of the second half of the nineteenth century, though it remains as relevant now as it was 100 years ago. Quantum mechanics, as studied by undergraduate chemists, is over 50 years old, but is likewise a vital ingredient of modern chemistry.

At the same time the subject itself has not stood still. So how does one teach both the old and the new, and how is the old kept fresh and stimulating? And then there is the problem of conflicting requirements. Scottish, English and North American universities all have different entry standards, and give courses in the first year that demand different prerequisites, so the author of a general text has to decide the potential market that is being addressed — it is impossible to please all of the people all of the time. The market for such broad-brush textbooks is enormous; most publishers have one or more appropriate titles in their lists, yet most of them are virtually indistinguishable in their approach and presentation.

Peter Atkins is well known as an author of physical chemistry textbooks. His *Physical Chemistry* (Oxford University Press, 1986) is now in its third edition and is widely used by relatively advanced undergraduates. Another of his books, *Molecules* (W.H. Freeman, 1987), presents the concepts of molecular shape, so important in biological aspects of chemistry, in a novel and entertaining way. Atkins also has strong views about the topics which he feels should be taught; in an article published in 1987 (*Chemistry in Britain* 23, 640–641), he argued the case for teaching through computer modelling and provoked considerable debate. We have, therefore, an experienced author with some controversial views — in turning to general chemistry, has he produced something different from the standard texts?

The initial impressions are encouraging. The book's presentation is attractive, with one-third of each page reserved for a column which displays diagrams, photographs and other figures. Colour is used

well, although the pale-blue background to photographs of apparatus sometimes obscures the colour changes that are being shown. The pictures of molecules are especially good, with space-filling models used as in *Molecules*. This new book must rank as one of the best-produced chemistry texts I have ever seen.

The content, however, is pretty conventional with none of the innovation that one might have hoped for. By British standards, organic chemistry is handled only superficially: there is a lightning tour of the subject scampering through everything from alkenes to nucleic acids in two chapters of 70 pages. There is no attempt to introduce mechanism — yet how can students ever make sense of organic chemistry without it? For inorganic chemistry there is a similar tour of the periodic table, and these two factors alone make it clear that a North American audience is what the author and publisher have in mind. As

one might expect, physical chemistry fares best; over two-thirds of the text is devoted to the subject, with a commensurate increase in the academic standard.

There are some good touches — a description of how to quote the correct number of significant figures in numerical calculations, and a massive number of problems and worked examples. Indeed, the book is (or will be) accompanied by a study guide, an instructor's manual, a solutions manual (only the odd numbered numerical exercises have answers in the book itself), a laboratory manual, a computer test bank, a video demonstration and overhead transparencies. My prognostication is that, in a very full market place, *General Chemistry* is likely to be a best-seller in North America but less used in Britain. □

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A closer look

Tom Mulvey

Principles of Electron Optics. By P.W. Hawkes and E. Kasper. Academic: 1989. Two volumes, pp. 1,188 plus indexes. Vol. 1 £37.50, \$84.50; vol. 2 £37.50, \$79.50; the two together £65, \$140. A third volume is in preparation.

It is 56 years since Ernst Ruska built the electron microscope that broke through the resolution barrier set by the optical microscope and later earned him the 1986 Nobel prize in physics. These instruments now have a huge influence on science and technology, diagnostic medicine, crime detection and food technology, and hence on society itself. The design of modern electron optical microscopes and the interpretation of the resulting atomic images is a scientific challenge. Electron microscopy makes great demands on theoretical physicists because it embraces the whole range of classical and wave optics, newtonian and relativistic mechanics, and quantum physics. Many former theoretical speculations such as 'quantum wells' can now be observed routinely.

A lot of books on the subject have appeared in the past, but *Principles of Electron Optics* is the first attempt, since the advent of the digital computer, to cover the entire subject systematically and critically. There are three volumes, each of about 600 pages. The first two, *Basic Geometrical Optics* and *Applied Geometrical Optics*, are reviewed here; Vol. 3, which will be concerned with wave optics, image interpretation and electron holography, is in preparation. The authors assume that the reader has a knowledge of physics and mathematics to

degree level, and aim to provide a self-contained, modern account of their subject for anyone "involved with electron beams of modest current density in the energy range up to a few mega-electronvolts".

In Vol. 1, the emphasis is on basic principles rather than instrumental applications. This, however, is no mere repetition of material to be found in textbooks — Hawkes and Kasper have reworked their material into a coherent and unified whole, making a careful appraisal of the best way to approach a given topic. Although the role of traditional analytical methods is clearly brought out, the impact of computer methods is rightly stressed. The authors compensate for some inevitable omissions by providing a comprehensive set of notes and further references directing the reader to the original literature. Volume 2 deals with the application of the theory to the calculation of the optical properties of lenses and other electron optical elements. Particularly valuable is the chapter on electron guns, a difficult and widely misunderstood subject.

This is a monumental and timely work — well researched, carefully proof-read, and marked by clarity of thought and expression. That it is in English and uses SI units is an enormous advantage for today's worldwide readership in electron optics and microscopy. It merits close study by all physicists and engineers concerned with the subject, and indeed by all those who wish to gain an insight into the power of theoretical physics to deal, often in a deceptively simple way, with the complex situations that now arise routinely in electron microscopy. □

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Collaboration in a wider Europe

Europe is in a mood to collaborate in research, as in much else, but doubts persist about the proper framework. This survey of science in Europe may resolve some issues.

EVERYBODY agrees that Europe is in transition, but the end-point is undefined. That is the starting point for the survey of science in Europe that occupies the following pages. Several important influences, cultural, economic and political, have conspired to provide an impetus for change that, in the long run, will help determine what the world is like, but the most interesting of them is the growing inclination of those who live in Europe to regard themselves as members of a community whose common interests transcend the chauvinistic differences that have bitterly divided them in the past.

That is why this survey differs markedly from others in this biannual eight-year series. It is not so much a record of achievement and disappointment in research, as of the mechanisms by which Europeans collaborate. And because there is so much going on, this will seem even more neglectful than previous surveys of important institutions contributing to the increasing cohesion of European science. It is hoped that those who are concerned with these institutions will not be too deeply offended.

Many of the institutions that now exist to foster European collaboration have their roots in the period immediately after the Second World War, and in the then common revulsion against the folly that had brought most European states economically to their knees. But there is an important sense in which the lesson also derives from the earlier 1914–18 war. In the settlement of that disaster, states called great powers redrew the map of Europe artificially while demanding that Germany should pay for the cost of the trouble it had caused — in retrospect a device for creating the conditions that made the Third Reich possible. That seemed a bad precedent in 1945. But by then, the great powers of the time had already (at Yalta) redrawn the European map so as to divide East from West. All the more necessary that the other ingredients of the settlement after 1918 should be avoided.

That is why the past 40 years have seen so many attempts in Western Europe to create a framework in which national interests and cultural differences would be subsumed within a common purpose. In rapid succession, there were formed the Western European Union (concerned with mutual defence), the Council of Europe (to foster common social and legal standards) and the succession of economic communities, which were at first concerned with commodities such as iron, steel and nuclear energy, but now, as with the twelve-member EEC, are concerned with the whole of economic activity. (The second half of this survey, beginning on page 726, deals with EEC science and what should be done about it.)

Collaboration is evidently infectious. The dramatic proof of that is Mr Mikhail Gorbachev's recent talk of the

"common European home". Gorbachev, who is not naïve, no doubt has many objectives. But suddenly, it seems, everyone wishes to collaborate. Poland, Hungary and Yugoslavia are talking of full membership of the Council of Europe, its Convention on Human Rights apparently not an obstacle. Turkey has applied for membership of the EEC, Austria is wondering whether to do so, while the Scandinavian countries and Switzerland may yet have a close relationship with the EEC.

Where will it end? And will the outcome revitalize European science? The continent (including its offshore islands) has never been full of optimists, which is why there is also now as great a need of realism. It is easier to decree collaboration than to ensure that it succeeds, especially in the practice of scholarship and research — and when the decrees are made by politicians. Here are some challenges and rules for optimists:

■ **Higher education.** In the 1860s, the great Ernst Mach left Vienna to teach at Prague, which is not very far away, but that would not now seem a natural move. Europe now has too many separate university systems. Even within the EEC, too many universities are too tightly in the pockets of their governments, national or even regional, yet there are no plans for integrating them. So how, and when, to make progress towards a wider framework?

■ **Basic research.** There are two frameworks of collaboration — within the EEC, and more extensive (typified by CERN and the European Science Foundation). Within the EEC, the European Commission should in future play a stronger part in supporting basic research (see page 734), but that may be detrimental to wider collaboration. How are those desirable goals to be balanced?

■ **Applied research.** The EEC centrally supports research intended to put its industry in better shape, but there are contradictions, not least that with the principle that successful industrial companies stand on their own feet, paying for their own research and development. EEC practice is also a potential impediment to wider economic collaboration. How will those issues be resolved?

In all this, there is a crucial issue too often overlooked — the danger that European collaboration in some defined framework will be exclusive of the world outside. The danger that the EEC will emerge from the upheaval planned for 1992 as a high-tariff chauvinistic group of self-satisfied states is the most immediate. Those who would suffer most would be those stranded within the tariff barriers, but Europe's relationship with the outside world would also be seriously damaged. That is why the EEC's goal of a single market, promised for 1992, is not in itself sufficient. It also matters crucially whether it is an outgoing grouping. □

Once-lame duck now exudes self-confidence

Paris

THE European Space Agency (ESA) is "the best example of what European collaboration can achieve", says its director-general, Reimar Lüst. "We are not only involved with basic science and new technology, but are faced with heavy competition, unlike the European fundamental research facilities, which have life easy."

Yet, with only one-tenth of the budget available to the US National Aeronautics and Space Administration (NASA), ESA has become a force to be reckoned with.

The collaboration began in the early 1960s, under the impetus of European scientists, as the European Space Research Organisation (ESRO) and the European Launcher Development Organisation (ELDO). ESRO was highly successful, developing a series of scientific satellites launched by NASA, and establishing specialized space-science centres in the Netherlands, Italy and West Germany. But ELDO was a damp squib and failed to produce a viable launcher.

ESRO and ELDO metamorphosed into ESA in 1975 with the signing of a convention by 11 European countries: Belgium, Denmark, West Germany, France, Ireland, Italy, the Netherlands, Spain, Sweden, Switzerland and the United Kingdom. Austria and Norway joined last year, when Finland became an associate member.

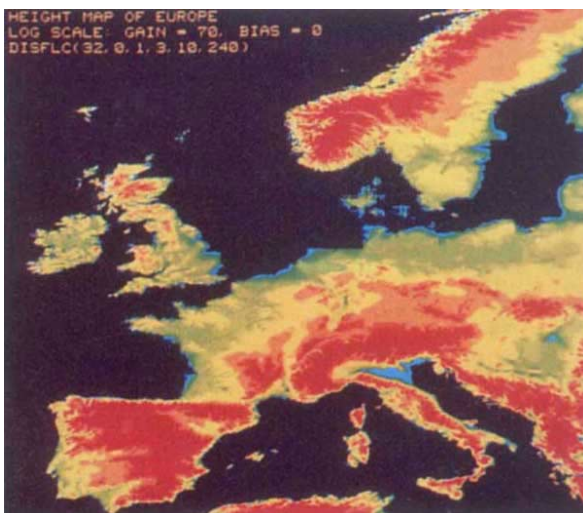
ESA has taken European space capability out of a purely scientific domain, dependent on US launchers, into telecommunications, Earth observation and meteorology. A major step was the establishment, in 1980, of Arianespace, a commercial joint-stock company (59 per cent owned by France) responsible for the exploitation of ESA's successive generations of Ariane rockets. With a record of only four failures out of 30 launches, Arianespace now has 50 per cent of the world commercial launch market.

Once the heart of European collaboration in space, basic science now represents only 12.4 per cent of ESA's total 1,573 million ECU budget. But the science programme, like the general budget, is mandatory. All member states have to contribute in proportion to their gross national product. Consequently, unanimity is required whenever policy or budget decisions are put to the vote at ministerial-level meetings. Last year,

Britain found itself isolated when it initially refused to agree a 5 per cent annual increase in the science programme budget, holding up scheduling and potentially threatening some projects.

The science programme is constructed around a long-term plan, put together by the science programme committee and covering the next 15 to 20 years. The current programme, called Horizon 2000, was jubilantly approved at the 1985 ESA council meeting at ministerial level, in Rome. It comprises four 'cornerstone' projects in solar terrestrial physics, a comet nucleus sample return mission, an X-ray multi-mirror mission and a sub-millimetre astronomy project.

For Lüst, the science projects often help the push towards new technology — as was the case with the optics of the Hipparcos astronomy satellite, one of



ESA's satellite-view of Europe. Europe could comfortably double spending on space, says ESA's director-general.

four 'medium-sized' missions planned under Horizon 2000. (Hipparcos is due to be launched in a few weeks.) Two others, Ulysses and the Space Telescope, are joint ventures with NASA, and have had to stay on ice for almost three years since the suspension of shuttle launches in 1986. Future ESA missions will be launched by its own Ariane rockets.

The applications programmes are where national passions, purses and fantasies find direct expression. Participation is optional, but ESA ensures that backing nations receive industrial contracts in proportion to their investment.

Telecommunications satellites have been supported by all member states and, with Earth observation and microgravity projects, have stimulated the European Commission to become involved to work out norms, organize networks of users and to improve what it considers to be an in-

adequate infrastructure.

But ESA's most controversial and expensive move, where tangible returns are less certain, came in November 1987 when a majority of member states backed a programme for manned space missions. Together, ESA's contribution of an attached laboratory to the NASA Freedom space station, a free-flying laboratory and a polar platform, now account for almost 40 per cent (609 million ECU) of its total budget, while the French-conceived Hermes space plane and the Ariane 5 rocket needed to launch it swallow a further 10 per cent (153 million ECU). Britain has opted out of these projects, so British companies will benefit hardly at all from the contracts.

Lüst applauds the decision to enter the manned space arena. "The trend will move towards robotics, but this will take 20-30 years. Meanwhile, there is still a need for people." He argues that the Hubble Space Telescope, due to be launched at the end of the year, has been designed to last for ten years. But if it needs repair during that time, "it may be more expensive to recover than to repair it in orbit."

To Lüst, there is no doubt about the value of ESA for European science and industry. "The system really works. On satellite projects, probably two dozen truly European teams work together. Europe needs a drive on high technology and ESA provides an excellent vehicle for this." He is proud that ESA scientists and engineers are already exploring new frontiers such as robotics, synthetic radar and laser applications for optical communication between satellites.

"We also need more civil and less military research", says Lüst. Since 1980, the ESA convention has declared the purpose of its activities as exclusively peaceful, a constraint that has strung out negotiations with NASA over the Freedom space station. He also feels that space science is good value for money. "Europe's gross national product is about three-quarters of that of the United States, yet we spend only one-eighth per capita of what the United States spends on civil research. Only 0.05 per cent goes on space technology. Even if the figure doubled it would still be all right."

But last year, ESA found its member states less united and resolute than in 1985 and is due for a new round of debate when it seeks backing for the Data Relay Satellite, which, according to Lüst, is an essential element of the Columbus package.

Meanwhile, Columbus is not yet out of the woods. President Bush has still to persuade Congress to go ahead with Freedom. But, says Lüst, if the United States reneges, ESA will continue alone, although it may be more reluctant to co-operate with NASA in the future.

Peter Coles

Southern sky surveyed

Garching, near Munich

CREATED out of the ruins of European astronomy after the Second World War with the help of the Ford Foundation, the European Southern Observatory (ESO) has become one of the most successful undertakings in European science. After years of solid contributions to optical astronomy, ESO now stands poised to seize the initiative internationally with its planned Very Large Telescope (VLT).

Following the model of the European nuclear research centre CERN, ESO goes beyond the financial abilities of individual member states to build powerful instrumentation in its field. Since the creation of ESO in 1962, its main tasks have been to build telescopes for scanning the Southern Hemisphere and to develop new instrumentation for optical astronomy.

Among telescope designers, ESO is now known as the pioneer of what are called active optics, embodied in its New Technology Telescope (NTT), which began a series of trial observations on a mountaintop in Chile in March. Active optics are the computer engineers' alternative to the cumbersome slabs of glass from which telescope mirrors have traditionally been made. Many hold that the diameter of conventional glass mirrors has now reached its technical limit, and that active optics are the only way forward.

The idea is that a microcomputer controls transducers which in turn control the precise figure of a thin glass mirror, distorted though that may be by gravity and other influences. The aim is superior resolution, but also telescopes that are more manageable and less costly. Active optics will play a pivotal role in the VLT, which is actually a set of four 8-m mirrors yielding the equivalent of a single 16-m dish. The telescope will be the largest in the world when it is completed in 1998.

The five original member states — Sweden, West Germany, Belgium, the Netherlands and France — were joined by Denmark in 1967 and Italy and Switzerland in 1982. Austria is now making a strong bid for membership. But despite an early interest, Britain has stayed out of ESO, concentrating at first on collaborations at observatories in South Africa and Australia and, more recently, on telescopes of which it is the chief owner in the Canary Islands and Hawaii.

ESO has its European headquarters in a smart modern building in the Bavarian flatlands at Garching, near the Max Planck Institute (MPI) for Physics and Astrophysics, the MPI for Plasma Physics and the campus of the Technical University of Munich. It operates or shares the 15 telescopes (counting the NTT) at La Silla in northern Chile, 600 km north of Santiago. The staff of 280 is divided

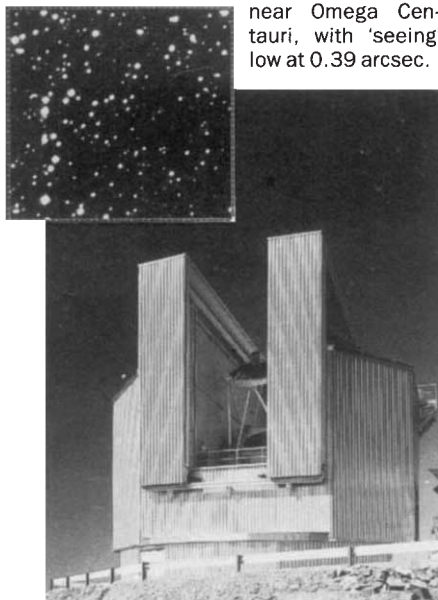
between Garching and La Silla, where most of the technical staff is Chilean. In Garching, there are about 50 astronomers (10 permanent) and 80 other staff members. The remote site at La Silla — Spanish for "the saddle" — in the Atacama Desert is "one of the most unpolluted sites on Earth in every sense", says Richard West, an ESO astronomer. The first telescope went into operation in 1968.

The organization of ESO is virtually identical to that of CERN (see page 722). An administrative council meets twice a year to decide budget and policy issues. A director-general (now Harry van der Laan of the Netherlands) is responsible for day-to-day operations. The scale is the most important difference: ESO is about ten times smaller than CERN. The annual budget of about DM50 million (it will rise to DM100 million in the 1990s because of the VLT) is shared by member states, with the larger states paying more.

ESO has generated excitement among European and outside astronomers with its ambitious plans for the VLT. Expatriate Europeans such as Jacques Beckers of the Netherlands, recently of the US National Optical Astronomy Observatory, have been lured back from the United States to work on the VLT, whose construction was approved unanimously by ESO member states in 1987. The VLT construction budget is DM382.2 million.

VLT is the logical offspring of NTT. The active optics of NTT are expected to allow the 3.58-m telescope a threefold improvement in resolution over that of previous instruments. Active optics will be a necessity for the VLT, whose 8-m mirrors would collapse under their own weight if ground at conventional thick-

The New Technology Telescope and results of 'first light' on 23 March. Inset shows an area near Omega Centauri, with 'seeing' low at 0.39 arcsec.



ness. ESO researchers expect to choose a site in Chile for the VLT by 1990. The first telescope may be working as early as 1995.

One of ESO's obvious difficulties is its popularity. As at other well-equipped observatories, only about a half of the 700–750 applications for observing time can be granted each year, and many successful applicants receive less time than they ask for. A committee made up of astronomers from each member state rates the proposals numerically, but the director-general has the final say. Van der Laan's task is "tricky and difficult", says West, especially because not all heavenly objects can be observed all the time.

Although ESO is a European organization, astronomers from member states enjoy no advantage in the refereeing process. Chilean, US and British groups have all been granted time in recent years. West says that the competition for time has raised the quality of European proposals — and, in the end, Europeans do receive most of the observing time.

What advantages accrue to ESO's member states? The most obvious is the organization's reason for existence — that astronomers have access to telescopes of a quality that national organizations would be unable or unwilling to finance. There are other tangible benefits, not least access to technical knowhow. Thus ESO was early in the field with the use of image-processing systems for extracting information from images of the heavens. Astronomers who used to travel to Garching to use its image-processing systems have often been able to find the funds with which to build their own, but ESO continues to provide software support to member states and others free of charge.

Italy and Denmark have decided to build versions of ESO instruments for their national astronomy programmes. Indeed, Italy joined ESO in part to gain the technology to build its own 4-m telescope. The project has a name — Galileo — but not yet a site.

ESO's small staff of professional astronomers, deliberately kept small, is regarded as a collective benefit. To keep ideas flowing in, there are only a few permanent positions. In striking a balance between a self-standing research organization and a service organization for its members, ESO (prodded by its council) has leant in the second direction.

ESO wins praise from astronomers not only for providing a unique opportunity for them to observe the southern sky. La Silla and Garching are also well known as meeting places where collaborations are incubated. VLT may be such a powerful magnet for observers everywhere in Europe that its influence could extend far beyond mere time-sharing. After all, as Beckers says, VLT "is where [optical] astronomy is going." **Steven Dickman**

Fight for tape recorders

Dwingeloo (Netherlands)

IMPROBABLY hidden in the pine-woods south of this hamlet near Groenningen is a radiotelescope and a smattering of low-built offices. The site is that of a European collaboration of radioastronomers who may yet be able to build a gigantic tape-recorder allowing a score of observatories elsewhere in Europe to pool their signals in the astronomers' common cause of seeing further. But the cause has been cruelly frustrated.

Dwingeloo is also the headquarters of the European Consortium for VLBI (for Very-Long-Baseline Interferometry). The technique of correlating accurately timed signals from several radiotelescopes, all pointing in the same direction, makes the accuracy of the position of a simultaneously observed radio source greater than the sum (in any sense) of the accuracy of the separate observations. The headquarters of the Netherlands Foundation for Radio Astronomy (NFRA) is also sited at Dwingeloo.

The European Consortium is, just now, at its wits' end because of uncertainty over its plans for the future. Last year, it applied to the Stimulation programme of the EEC for funds to finance its technical development. But the EEC Commission said "No".

In reality, the VLBI Consortium is a club. The founder members are the observatories at Bologna, Effelsburg (near Bonn), Dwingeloo, Jodrell Bank and Onsala (Sweden), and associate members include the Paris Observatory (Meudon) and institutes in Moscow and Poland. The six regular collaborators have committed between a quarter and a third of their observing time to VLBI. Projects are put forward by astronomers individually, and assessed by a programme committee. Richard Schilizzi, project manager for NFRA at Dwingeloo, says that the consortium is two to three times oversubscribed on a 24 hours a day basis. Moreover, the network is growing rapidly.

Like all VLBI collaborations, the club's collective ambition is so to improve on the accuracy with which measurements of the position of distant radio sources are made that the large-scale yardstick of the structure of the Universe will be better known, nearby galactic and even stellar phenomena will be better understood and the year-by-year movement of the Earth's tectonic plates will be more accurately measured.

The European Consortium is anxious not to be left behind by its competitors, the chief of which is that in the United States. Europeans are envious of the US VLBA (Very-Long-Baseline Array) project, which is now building ten 25-m dedicated radiotelescopes operating in

nine frequency bands between 1 metre and a few millimetres.

From the outset, VLBI techniques have been especially valuable in mapping the structure of distant quasars and other active galaxies; Schilizzi says the recent observation by VLBI of extragalactic water and hydroxyl masers offers a chance of constructing an independent and objective distance scale for the Universe.

So, with all that promise, why are people so glum? The difficulty is that the analysis of VLBI data is becoming steadily more complex — and expensive. Collaboration requires that each observatory should have a decent clock and a more than decent tape-recorder.

With the development of millimetre-wavelength telescopes (which improves the resolution of the system), recorders must be capable of storing huge amounts of data — 512 megabits a second is the new standard, which is roughly the information content of 100 standard video signals. But then it is necessary centrally to correlate up to 20 of these recorded signals electronically, which means synchronizing and then multiplying together each pair of signals from the contributing observatories.

The 18 million ECU for which the European Consortium applied last year would have been spent on the development of the tape-recorders and the correlator followed by their installation at Dwingeloo. The consortium points out that the only correlator in Europe at present capable of dealing with millimetre-wavelength signals is that at Effelsburg, which can handle signals from only three independent telescopes and which is, in any case, fully extended.

Schilizzi says that it is essential that the project should cover the cost of providing each of 11 telescopes with a tape-recorder (and a spare). Otherwise, he says, national grant-making agencies would skimp on the cost, and the resulting non-standard equipment would yield signals that could not easily be compared.

There is a Euro-industry argument on the consortium's side: broad-band recording techniques and equipment will be in great demand as high-definition television gets under way. So why not put some millions of ECUs into the development of such machines, equip a score of observatories with them more cheaply than they could be bought and leave some European manufacturer with an expertise now lacking?

It seems generally agreed, even in Brussels, that the argument is appealing. People have plainly listened to it attentively — and have then decided that there is nothing they can do. But the last has not been heard of Dwingeloo's ambitions. □

Monastic calm and money worries

Paris

JUST a 20-minute train journey from the centre of Paris in 11 hectares of parkland lies what has been described by its director, Marcel Berger, as "a monastery and a beehive" — the internationally renowned Institut des Hautes Etudes Scientifiques (IHES). The paradoxical metaphor is justified.

On the one hand, the institute provides the isolated calm necessary for profound meditation on theoretical problems, while the six permanent fellows last year attracted 872 researchers from 42 countries to exchange ideas in the vanguard of mathematics and theoretical physics.

Inspired by the Institute for Advanced Study at Princeton, IHES was set up in 1958 by Léon Motchane, Jean Dieudonné and Robert Oppenheimer as a European centre of excellence in mathematics and theoretical physics. The Second World War had seen some of Europe's most able scientists seek refuge in the United States; naturally, there were fears that the centre of gravity of science would move irretrievably across the Atlantic. In poetic justice, IHES stands on the site of a chateau commandeered by the Nazis during the occupation.

Very much like its Princeton model, IHES has a small number of permanent fellows on comfortable stipends for life — although mathematician René Thom is the first to continue to retirement so far, becoming an honorary fellow this year.

Fellows are free from all teaching and administrative obligations and do not have to account for their time, apart from an undertaking to spend 6 months of the year at the institute. In practice, they have catalysed revolutions in their specialisms. During the 1960s and 1970s, for example, the institute established itself as the world's temple of algebraic geometry, through Alexandre Grothendieck, Jean Dieudonné and Pierre Deligne.

Although fellows leave an indelible mark on the choice of themes, these have evolved over the years, aided by the presence of a handful of invited long-term visitors who stay for around five years, and a long line of visitors spending up to a year at the institute.

Today, the permanent fellows are Jean Bourgain, Mikhael Gromov, Louis Michel, David Ruelle and Dennis Sullivan. This year, theoretical physicist Thibault Damour fills a vacancy left by Oscar Lanford, opening a new avenue of research in general relativity and cosmology.

The institute's private status is anomalous, for 64 per cent of its FF19,287,000

SYNCHROTRON RADIATION

Grenoble as physics city

(\$3.2 million) budget comes from the French Ministry of Education — an initiative started by President Georges Pompidou in 1965. Before that, IHES scraped along with relatively small subscriptions from a handful of business sponsors. But this year, only FF570,000 (\$95,000) has been contributed by the private sector. Now, laments Marcel Berger, it is almost impossible to find money for basic research in industry. This has always depended on personal contacts established by the incumbent president, and can lapse when there is a change at the top.

Although genuinely international, IHES derives only 15 per cent of its income from European governments and is not eligible for European Commission support. Since 1971, the British Science and Engineering Research Council (SERC) and the West German Max Planck Gesellschaft have each given grants, this year of just over FF1 million (\$166,000). The Swiss and Belgian governments have also supported IHES since 1971, together providing about 5 per cent of its income, while the governments of Ireland, Denmark, the Netherlands, Finland, Portugal, Israel and Brazil also make modest contributions (between \$2,000 and \$13,000). The US National Science Foundation (NSF) has supported IHES since 1985, this year giving \$65,000. This grant, a rare gesture for NSF, reflects the large number of US visitors to IHES, by far the majority.

But money is becoming an increasing worry for the institute, which started the year in deficit. "The private status of IHES gives us great flexibility and autonomy", says Marcel Berger. "The fellows' stipends, while they cannot match those available in the United States, aim to free them from money worries, while it is possible to organize a seminar or arrange a visit at the drop of a hat."

But, with the library — a former music room — cracking under the weight of its books and needing expensive underpinning, Berger is having to buy time by leaving vacancies unfilled. An important ally has been found in the European Science Foundation which, since 1976, has carried out a five-yearly review of the IHES scientific programme. The panel's review helps guarantee the institute's autonomy, while ensuring that its high standards and international connections are maintained. That, in turn, makes governments — and, in principle, industry — happier to continue their support.

Berger says it is "unthinkable" that IHES would ever be threatened with closure. But if any of its sponsors, such as SERC, should be prevented by national government policy from helping, would suffer and its sometimes fragile occupants could once again become an endangered species in Europe.

P.C.

Paris

THE French city of Grenoble, near the Swiss and Italian borders, is, by a mixture of fate and design, becoming a 'Eurocity'. But unlike Brussels, Strasbourg or Luxembourg, Grenoble's cosmopolitanism stems not from politics but from a distinctive blend of unrivalled basic science facilities and new technology industry. The city's fourteenth-century university may already have had an international flavour, but twentieth-century physics has put Grenoble on the map of European science.

In 1946, the French national science research centre (CNRS) opened its first major laboratory outside Paris in the city, with its Service National des Champs Intenses. In 1956, the French atomic energy commission (CEA) chose Grenoble for its research establishment and in 1966 the Institut Laue-Langevin, which houses the world's highest-performance neutron-beam reactor, joined the family.

This year, construction has started on the latest, and most European, of Grenoble's big science instruments — the European Synchrotron Radiation Facility (ESRF). The idea that Europe should build its own major synchrotron facility was first put to the European Science Foundation in 1975. There followed a period of diplomacy, definition and costing until, in 1985, the two major promoters, France and West Germany, proposed to go ahead with the project.

At the end of 1985, a memorandum of understanding was drawn up between France, Germany, Great Britain, Italy and Spain. In 1987 the protocol was signed, with Switzerland and a Nordic consortium joining the original five members. The formal convention and statutes allowing money to be spent was signed in Paris on 16 December last year.

At present, the site is being excavated, but, says Karl Witte, assistant to ESRF's director (Ruprecht Haensel), "you can already see a few circles on the ground". The first seven of ESRF's beam lines are due to be opened in 1994, with a target date of 1998 for the full 30 planned. When completed, ESRF will be the world's most powerful radiation synchrotron, able to accelerate electrons or positrons up to 6 GeV, with a beam width of about one tenth of a millimetre.

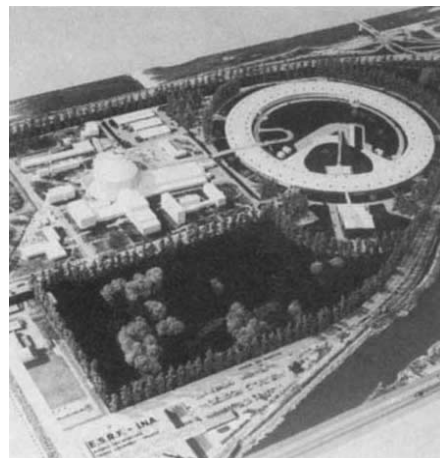
But the title may quickly be lost. A 7 GeV synchrotron is to be built at the Argonne National Laboratory in Illinois and, last December, the Japanese government announced plans for a 8 GeV synchrotron facility near Osaka.

The total cost of ESRF will be FF2,600 million (about \$406 million), with operating costs for 1994–98 estimated at FF1,000 million. France is contributing 34 per cent of the cost, and in addition has

supplied the site and the local infrastructure. West Germany is the second largest partner, with a 24 per cent stake, followed by Italy (14.5 per cent) and Britain (12.5 per cent). The balance will be shared between Spain, Switzerland and the Nordic countries (4 per cent each) with Belgium providing 3 per cent.

Last month, at the first ESRF users' meeting, 200 Europeans and a handful of colleagues from the United States, Japan and Israel, discussed priorities for the first seven beam lines and verified that the synchrotron's specification (called the 'red book') is still appropriate. Discussions will continue until the scientific advisory committee meeting at the end of June.

Although ESRF still has only a skeleton staff, there are already signs that it may be difficult to achieve a mix of nationalities in the resident staff (on five-year fixed con-



A model institute, but building is under way.

tracts) which reflects members' relative contributions. "We wanted to keep the French presence below 50 per cent", says Witte, but the proportion seems now to be about 52 per cent. The difficulty is that it is not easy to attract scientists and technicians from other member states because ESRF can offer only French salaries in line with those of the CEA. Constitutionally, ESRF is a French company and in that respect unlike, for example, CERN, where salaries are fixed on the international European scale.

More than 2,000 people are expected to use ESRF each year, with all costs paid for out of member states' contributions. Experiments will be selected on the basis of merit by a scientific panel on which each member state will be represented. If a "significant imbalance" emerges between a country's rate of use of the facility and its percentage contribution, the statutes provide for an eventual retrospective "readjustment" of payments. This principle may free referees assessing proposals for using the machine from the straitjacket of national time-sharing quotas.

P.C.

Europe's most influential and costly collaboration

Geneva

CERN, the European Laboratory for Particle Physics whose new name does not match its old acronym, seems always to be celebrating something. A few years ago, it was the successful production and identification of the particles called the Z^0 and the $W^\pm$ whose existence put the cap on the unified theory of electromagnetic and weak nuclear forces due severally to Salam and Weinberg.

This year, CERN will be celebrating the commissioning of its latest accelerator, called LEP (for large electron-positron storage ring).

With just a few months to go to completion, LEP seems already assured of success. The tunnel (with a circumference of 27 km) has long since been complete and the magnets which keep the two counter-circulating beams installed. One section of the storage ring has been tested for fidelity with real electrons. The hope now is that it will be possible to put electrons and positrons into circulation soon after midsummer and that the first physics will be done early in the autumn.

The most striking feature of the way that people talk about this prospect is their confidence. The underlying assumption is that every project to build a particle accelerator that CERN touches turns out successfully. Certainly this has been the laboratory's record since the late J.B. Adams, as director, built the machine called the Super-Proton Synchrotron (SPS) and began on the task of building LEP.

The immediate objective is to collide beams of electrons and positrons circulating in opposite directions in the same vacuum chamber and each carrying 55 GeV of energy. Apart from the gigantic scale of the construction, the technical difficulties are those of shaping the counter-circulating beams of electrons and positrons and controlling the position of the particles both in space and time. The particles travel in bunches and are

meant to coincide in space and time at the eight points around the ring at which collisions are meant to take place.

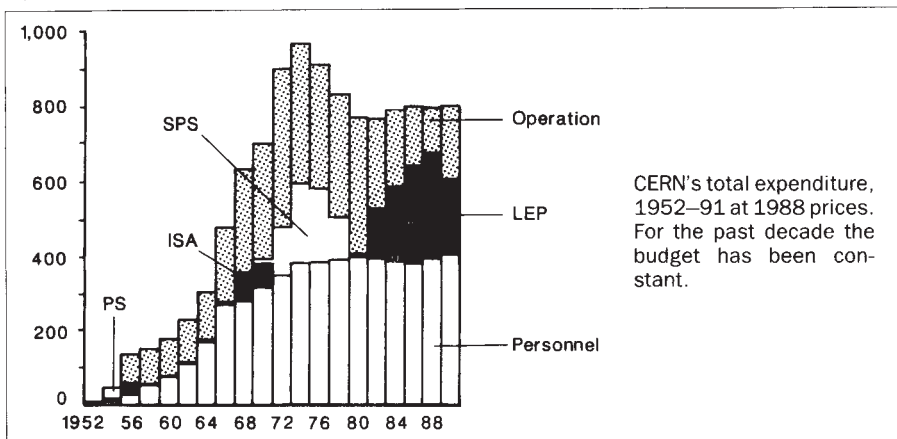
With this energy, colliding pairs of electrons and positrons should yield copious numbers of the heavy bosons required by the electroweak theory, allowing the properties of these particles to be defined in detail. But there is also a scheme for making a more powerful machine of LEP by substituting superconducting cavity oscillators for those now meant to supply LEP with power. The result would be, perhaps by 1995, a machine capable of colliding 100 GeV electrons and positrons.

The scale of this project is quite breathtaking. The surface of the site shows no sign of the storage ring, which is in places

be a formal examination of continued British membership of the international high-energy physics community (carried out by a group under Sir John Kendrew), a further examination under the eye of the CERN council of the efficiency of its operations and, finally, a decision that British membership would continue, at least for the time being.

It is understandable that the workforce at Geneva (about 3,500 strong) should feel slighted by these signs of less than full approval. They are quick to point out that, as high-energy physics laboratories go, CERN is cheap, with a budget of just over 800 million Swiss francs a year out of which it must pay for the physical cost of building LEP (roughly SFr1,200 million since construction began in 1981).

In reality (see figure), CERN has been required to live within a fixed budget since the beginning of this decade. In the process, it has become expert at cannibalizing its existing machines. Electrons and positrons



100 metres or so underground despite the way the ring has been tilted from the horizontal (to minimize excavation costs). Getting to a particular experimental hall requires a journey in a vehicle through the rural lanes of (mostly) France and Switzerland, where agriculture continues undisturbed.

The hall that houses the planned experiment called Delphi is really an underground cavern 30 metres high which, last month, was buzzing with people threading power and data cables through the proper positions in the harnesses designed for them as if they were acrobats building a cocoon of cable for a mechanical monster lost underground (see this week's cover). Half-way up the detector being assembled are the ports that will deliver coincident bunches of electrons and positrons simultaneously to the centre of the detector.

That is the heroic face of CERN, which is also, by being the most costly of all international collaboration in Europe, also one of the most controversial. More accurately, that has been how it has seemed from Britain in the past few years.

In 1984, it was decided that there should

for LEP, for example, will be given their high energy by first circulating them through the oldest machine in operation at the site (a proton synchrotron) and then through the SPS (giving them a total energy of 20 GeV) before they are fed into the larger LEP ring for final acceleration and storage.

A bizarre prospect for the more distant future is that, at some stage, the same cascade of accelerators may also be used for the purpose for which they were designed (accelerating protons or antiprotons) with which to fill a companion hadron storage ring in the same LEP tunnel. Since the two booster synchrotrons also have other functions on the site, the planning of what kinds of particles are delivered to which experimental rigs in what sequence threatens to become a planners' nightmare.

The Large Hadron Collider, the scheme for equipping the LEP tunnel with a second circular vacuum chamber for storing counter-circulating protons and antiprotons, is for the time being only a possibility. For one thing, the 14 full member states have not approved the scheme (which, with its superconducting

Science in Europe

THIS brief survey of collaborative research in Western Europe has been compiled by Steven Dickman (Munich Correspondent), Peter Coles (Paris Correspondent), Peter Newmark (Deputy Editor), whose contributions are attributed, and John Maddox (Editor).

The objective has been to illustrate by means of examples the manner in which collaborative research in Europe is undertaken rather than to provide a comprehensive survey of the institutions involved, and of their objectives. □

magnets, would cost "1.5 LEP").

In any case, physicists would prefer to see what LEP itself produces before linking their ambitions to a particular successor. Some choices, the possibility of building opposed electron and positron colliders, for example, might make it necessary to leave the cramped site at Geneva.

On the face of things, CERN can thus see the way forward until the end of the century, which will amount to roughly half a century after its foundation (at a meeting organized by UNESCO in 1951). What, by then will it have accomplished?

Judgements of this kind are necessarily subjective. From the start, CERN has been most notably a European citadel of technical expertise, both in the construction of scientific instruments on a huge scale and in the development of fast data networks and communications interfaces for the handling of the data it produces.

While there is a sense in which CERN would be a pale shadow of its present self

CARLO RUBBIA

New machinery on the horizon?

Geneva

CARLO Rubbia is a tall man with a stoop who became director of CERN at the beginning of the year, after helping to put the institution on the map by finding the intermediate heavy bosons (and being awarded the Nobel prize for physics in 1987). He seems to have thrown himself into his new job with high-energy diplomacy, talking to the high-energy physics communities elsewhere, notably in the United States.

Rubbia is in no doubt that CERN is technically more excellent at the craft of building particle accelerators than either the United States or the Soviet Union. He points to the high estimated cost of building the SCSC in Texas, probably in excess of \$4,500 million, and to the difficulties (now resolved) of grafting onto the Stanford Linear Accelerator a device for colliding electrons at high energy.

But Rubbia also acknowledges that accelerators cannot keep on growing as they have been, and that the time must come when accelerators are built in a wider framework than in the past. Whence the diplomacy.

The strategy is to keep CERN in the forefront of high-energy physics while arranging that member states in Europe do not have to meet all the extra cost. Rubbia claims that Geneva has the world's only permanent team of accelerator designers and builders whose success has been established by demonstration. Will not others wish to join in collaborations with them, not just for physics but for construction?

One intriguing possibility is that future members in large consortia might make contributions in kind to future projects. There is even talk that the Soviet high-energy physics laboratory at Serpukhov,

had it not been for the discovery of the Z^0 and the $W^\pm$ mesons, that slights the importance of the growing range of general physics that pours out of Geneva each year, from the precise determination of the gyromagnetic ratio to the fabrication of atoms of antimatter.

Luck has helped. At the outset, with nuclear physics riding high and not easily distinguished from high-energy physics, CERN enjoyed the enthusiastic backing of powerful figures in Britain, France, West Germany and Italy. But by being consistently more cosmopolitan than its membership (with deliberate policies of forging links with Soviet and US physics), it has won and kept a place in international high-energy physics. In the process, it has become a free-standing centre of excellence whose connections run through the whole of Europe. There are many projects on which Europe spends 800 million francs a year without anything like the benefit. □

which is already fabricating superconducting magnets for its own new proton accelerator, might carry on making magnets for the Large Hadron Collider at CERN when its own needs have been met. Rubbia will make partnerships with anybody, on any favourable terms, to see the next generation under way.

But what would happen CERN if good sense required that the next machine should be built, perhaps by a larger consor-



Rubbia stresses CERN's ability to build accelerators.

tium, elsewhere? Rubbia notes that the present machines will have life left in them at the turn of the century, and that diversifying of present interests could also provide continuity on the Geneva site. But that is Rubbia's cautious side. The chances that he will be making a fierce fight for some new machine at Geneva a few years from now. □

ESF

Small but a powerful membership

London

THE European Science Foundation (ESF), based in Strasbourg, is the smallest but one of the most interesting of all European collaborations. It has a budget of its own amounting to less than FF14 million a year, but its influence is much greater than its budget would normally command.

The explanation is that ESF's members are neither people nor governments, but public grant-making agencies in European states. From West Germany, for example, both the Max-Planck Gesellschaft and the Deutsche Forschungsgemeinschaft (DFG) belong. All five of the British research councils as well as the Royal Society are members. Altogether, there are 50 members from 18 European countries, Finland and Turkey included.

In practice, what this means is that a project which is backed in Strasbourg is likely to win a sympathetic hearing when its advocates later take the hat around asking for substantial funds to supplement whatever seed money that ESF may have provided.

Perhaps the most striking illustration of this successful way of working is the launching of the European Synchrotron Radiation facility, now being built at Grenoble (page 721). The project first surfaced about a decade ago as a recommendation of an ESF committee consisting of accelerator physicists and potential users.

ESF knew the cost would be beyond its scope, but reckoned that it could carry out the diplomacy required to build a consortium of its grant-making members to launch the project, which has now successfully been done.

Another ESF-originated programme is the European Geotraverse, a project for the geophysical investigation of the Earth's crust along a line from Scandinavia to north Africa.

Latterly, ESF has been spending much of its budget on supporting networks of research with funds with which to organize meetings and regular visits among themselves. At the same time, it appears to have accepted that the time has come to grow. ESF's director, economist Michael Posner, is working on a "forward look" that would multiply ESF's budget by three or even five.

The foundation has also appointed a new president to take over from Professor Eugen Seibold, whose term ends in 1990. Professor Umberto Colombo, chairman of Italy's Nuclear Energy Agency, will take over in November 1990. □

One of the better models of European cooperation

Heidelberg

LENNART Philipson, an imposing Swede, is particularly proud of 'The Operon', the new 320-seat auditorium at the European Molecular Biology Laboratory (EMBL). It symbolizes his drive, as director general of EMBL since 1982, to fulfil one of the original aims of the 15-year-old laboratory — that it should be a European centre for teaching and training in molecular biology, particularly for the benefit of the smaller countries that help pay the cost.

Training, however, was only one of the main roles foreseen for EMBL by those who argued 20 years ago for its foundation. They also claimed it would act as a meeting-place for senior European molecular biologists (as Cold Spring Harbor does for US biologists and CERN for European particle physicists), a provider of specialized facilities that would be beyond the means of most European countries (again like CERN), and a focus for molecular biology that could help to disseminate the techniques throughout Europe. Critics at the time argued that specialized facilities and large laboratories would funnel molecular biology and its practitioners into an elitist group of little influence on the development of the science in Europe.

With its training role to the foremost, EMBL now hosts annually a dozen practical training courses, most of them run by its own staff, and takes on about ten predoctoral students, whose quality is said to be exceptional. They take their places alongside the 120 or so postdocs who are present at any time, in a laboratory that is demonstrably European to judge by the mix of nationalities of the scientific staff.

But Philipson argues that the training functions of EMBL are much more extensive. With almost all the scientists employed on short- or medium-term contracts, there is no elitist group. Rather, youngish scientists are given an opportunity to make their scientific mark during their most productive years, before dispersing, often to their country of origin, as trained leaders with a European attitude.

The other side of that coin is less bright: with such a turnover of even its better-known scientific staff, EMBL has some difficulty in building up and sustaining a reputation as an outstanding laboratory, which is what many of its critics think it should be for its size — about 500 people, of whom half are on the laboratory staff — and cost — now about £17 million a year.

At times, this has led one or other of the countries that foot the bill for EMBL to threaten to withdraw. The biggest threat, which ran throughout 1983, came from Britain, but ended when a Medical

Research Council review of the laboratory concluded that the British contribution could not have purchased equivalent work of equal quality within Britain.

In fact, no country has withdrawn its support from EMBL, and several more have joined in recent years, although Ireland, Iceland, Portugal and Belgium remain unpersuaded. Of these, only the Belgian contribution would make a noticeable addition to EMBL's budget. If Belgium joined there would be funds to occupy the last remaining space at EMBL — a floor of the brand new wing that houses 'The Operon'.

The same wing already houses the Bio-computing Programme, which recently became the seventh research programme at EMBL and which is, in many ways, just the kind of activity in which a European laboratory should be engaged.

On the one hand, it is an attempt to build a substantial research programme in an area of science — theoretical and computational biology, particularly in relationship to proteins and nucleic acids — in which Europe needs urgently to invest. But that is why it is disconcerting that EMBL is finding serious difficulties in recruiting experienced group leaders.

On the other hand, the Biocomputing Programme includes the EMBL Data Library, which shares with its US and Japanese counterparts the unenviable tasks of feeding emerging DNA sequences (27 million bases so far) into a database and then making it accessible worldwide. The former task is being eased because sequences are increasingly being deposited directly in the library, and the latter by the advent of EMBnet, which will be able to provide access to the current library through national European computer nodes on a DecNet-based network.

Even so, the accelerating rate at which new DNA sequences are being produced means that EMBL will not for long be able to support or even house the data library. Already, the European Commission supports 40 per cent of the activity, but only for two years in the first place, and Philipson is pushing for the foundation of a separate European institute (of bioinformatics, or some such) eventually to handle the library and its functions.

Of EMBL's other research programmes, those known as Gene Expression, Differentiation and, especially, Cell Biology have established reasonable international reputations. The Biological Structures programme, while still struggling to achieve equal footing, is said to be in better shape than it has been.

Meanwhile, the Physical Instrumentation and Biochemical Instrumentation

programmes are in danger of disappearing as a matter of policy. Stemming from the original conception of EMBL as molecular biology's counterpart to CERN, the development of instruments has always been one of its missions. Pursued somewhat in a vacuum by the founding director, (now Sir) John Kendrew, instrument development has been much more closely tied to biological programmes by Philipson, who now plans to integrate them completely.

This plan, like other major policy changes at EMBL, is subject to the approval of the laboratory's governing body, the council, which comprises delegates from each contributing country and is advised by a scientific committee. As director general, Philipson is responsible for planning and execution of approved scientific programmes at EMBL, assisted by a committee of staff members.

EMBL also operates two outstations, both with an uncertain future. For that at the Institut Laue Langevin in Grenoble (see page 723) the problem is that neutrons have not turned out to be particularly useful for biologists. Grenoble, however, is the site chosen for construction of ESRF (the European Synchrotron Radiation Facility, see page 723), which will be much more useful, and where EMBL has been asked to provide a biological research support laboratory. If EMBL agrees, the days (or, rather, years) of the second outstation at DESY (the Deutsches Elektronen-Synchrotron) in Hamburg will be numbered.

Until a few months ago, Philipson had not expected these problems to be his. But after the pathetic failure of the EMBL council to find a new director (see *Nature* 337, 397 and 589; 1989), he will stay until 1995 rather than leave in a year's time.

Perhaps for that reason, and tired of being on the defensive about EMBL as well as confident that the laboratory provides much better value for money than Community programmes, Philipson is now strongly promoting the idea of founding more EMBLs, for example, one devoted to plant biology and another to neurobiology.

Peter Newmark

Top men at EMBL (Philipson, left) and EMBO (Tooze).



EMBO

Workshops to postdocs and publishing

Heidelberg

IN contrast with EMBL, it is hard to find anything but praise for its original parent, the European Molecular Biology Organization (EMBO), which distributes fellowships, finances workshops and practical courses and now produces the *EMBO Journal*.

EMBO celebrates its silver jubilee this year. Its first grant was a small one from Israel, which to this day qualifies as a European state both for EMBO and EMBL. That was followed by a substantial grant from the Volkswagen Foundation. Finally, in 1969, an intergovernmental agreement established the European Molecular Biology Conference, the body through which EMBO's (but not EMBL's) funds are still provided. With Portugal soon to join and Belgium contributing unofficially, EMBO has a full house, and an annual budget of nearly £4 million.

The membership is drawn, somewhat unselectively, from the ranks of European molecular biologists. But the keys to its success are the select committees and reviewers, who decide which applications to support, and John Tooze, EMBO's executive secretary. Tooze also manages to edit *EMBO Journal*, act as scientific co-ordinator for EMBL and carry out research (four papers in *J. Cell Biol.* in 1987).

The greatest pressure on EMBO's budget is for long-term fellowships supporting postdoctoral fellows for 1–2 years in a country other than their own. Demand for these fellowships has increased greatly in the past five years, but plans to meet it were somewhat thwarted by British penny-saving in 1986, when EMBO's existence was guaranteed until 1995 and its budget set until 1991.

As a result, only about 30 per cent of the new applications for long-term fellowships could be financed last year, while about 40 per cent deserved support, says Tooze. Nevertheless, 142 new awards were made, together with 69 renewals of one-year awards for a second year.

Awards, Tooze emphasizes, are made strictly according to the quality of the application. There is no attempt to distribute some of the awards to each of the countries contributing to EMBO's budget — last year, 40 per cent of the new long-term EMBO fellows were working in US laboratories.

Of the original plans for EMBO, only the idea that it should award research grants remains unfulfilled. There are those who believe the time is right to start pressing for EMBO to be allowed to build on its success in that way. **P.N.**

HUBERT CURIEN

Portrait of a European

Paris

HUBERT Curien is a European enthusiast. Perhaps more than anyone, this modest 65-year-old French physicist, who is also research minister in the Socialist French government, has accompanied European science and technology in its trajectory from a collection of national initiatives towards true collaboration.

The European Science Foundation (ESF) and the European Space Agency (ESA), along with the European Commission's EUREKA and PACE programmes, CODEST and, most recently the Academia Europaea, have each benefited from his innovative flair and enthusiasm.

Although he has been president of both ESA and ESF, Curien was first of all an

IMAGE
UNAVAILABLE
FOR COPYRIGHT
REASONS

Curien — urbane, optimistic and idealistic.

ambassador for French science through his research on crystals and even has a new mineral, 'curienite', named after him. He has been director of the French national science research centre (CNRS) and president of the French national space research centre (CNES). He became Minister for Research and Technology in the 1984 Fabius government until the 1986 elections brought the opposition conservative party to power. In June last year, he once again became Minister for Research and Technology in the new Rocard administration.

Looking back on the past 30 years, Curien acknowledges that personal contacts between scientists inspired the creation of the first European organizations. "European countries did not know each other well in the 1960s", he says. So it was as a result of informal contacts between colleagues such as Curien, Lord Flowers and Reimar Lüst that the European Science Foundation and ESA got off the ground. "Now", he says, "we know each other better" and there is a European synergy arising from the various organizations and forums that have been established.

Historically it was through the creation of joint large scientific instruments, such as CERN, that European scientists found they could buy their own cake and eat it,

without having to be guests at the table of US colleagues. Other examples have followed, including the European Southern Observatory and the European Synchrotron Radiation Facility. But for Curien, European collaboration in science and technology means more than sharing expensive facilities. It is also a matter of bringing scientists together, not only to exchange ideas, but to influence policy.

"If thinking comes from the bottom and the money from the top, people at the bottom find themselves in a very happy situation, and as it is the people at the bottom who do the work, it is better if they are heard", Curien said in his keynote address at the foundation of the European Academy last year. And this 'bottom up' approach is the hallmark of initiatives in which he has played a central role.

EUREKA, Curien's brainchild, provides for trans-frontier technology collaboration where the projects are marketed. Similarly, CODEST, the European Commission's (EC) Committee for European Development of Science and Technology, is a platform for specialists to influence policy, while the proposed EC Assembly, due to be debated this year, will give 200 academics a voice in Brussels.

Perhaps because his own roots are still in the laboratory, Curien knows that the whole is also composed of parts which can easily find themselves isolated. It was he who first proposed the idea of 'networks' of laboratories as an alternative to the 'lame-duck' idea of European centres of excellence. Since 1974, 20 networks have been set up in specific disciplines, largely through ESF.

Now, as Europe finds its stride, Curien sees a danger that countries such as Greece and Portugal will find it difficult to keep up. "European cohesion can easily be opposed to excellence", he says. In areas at the vanguard of science and technology, he says, poorer countries may lack the facilities or trained researchers to take part. But if Europe opts for cohesion and drops these projects, the best are brought down to the level of the least good. For Curien, one way to achieve cohesion and excellence is through education.

The EuroPACE satellite teaching programme for continuing education, of which he is president, is one means to achieve equality of skills. He also wants to see more part-time posts in universities to allow researchers from poorer countries to keep a foot in good laboratories while raising standards at home.

Urbane, optimistic and idealistic, Curien continues to seek ways in which European collaboration can improve. "We cannot imagine Europe continuing as it is now. It must be more coherent." But, he adds, "with patience you can always get what you want". **P.C.**

Ministère de la Recherche et de la Technologie

What kind of bureaucracy is Brussels?

Brussels

EUROPEAN science is scattered over a score of countries: why look for it in Brussels? Because the European Commission, the executive arm of the European Economic Community (EEC), is there and has an increasingly important if controversial influence on the shape of things.

But who wants European science to be run by a bureaucracy? The question is often asked, much to the distress of the Commission's staff, whose members believe "bureaucrat" to be a pejorative term. This brief constitutional history of the EEC may put the question in a broader context although without altogether removing the slur.

The Treaty of Rome, signed in 1957, on the model of the US Constitution, defines a kind of European government with three arms — an executive (or administration) which is the Commission, a supreme court (the European Court of Justice) and a legislature (the equivalent of the US Congress). But the likeness between Brussels and Washington is only formal, chiefly because the political equivalent in Europe of the US Congress is a bastard blend of representation by member governments and by the directly elected European Parliament (which is not in Brussels at all, but at Strasbourg or Luxembourg according to the season of the year).

Much of the long-standing and broad argument about the future of the EEC turns on questions of how the Commission should be controlled politically. At the outset, the Commission was the servant of the Council of Ministers, which for practical purposes is a committee of national ministers chosen (by the governments they represent) according to the nature of the issue to be decided — agriculture ministers decide agricultural issues; prime ministers or their equivalents (plus retinues) attend the biannual meetings of the council proper, organized by whichever country has held the chairmanship of the council for the elapsing half year.

Originally, the council and its sub-councils were expected to reach decisions by consensus, which meant that each national government could veto the wishes of its fellow-members. Now, under the European Single Act of 1986 (whose most important effect is to promise the operation of a genuinely common market by the end of 1992), consensus is required only on decisions of a largely constitutional character (including the extension of EEC membership).

The sphere of influence of the European Parliament is still evolving, but is

comparable with that of the House of Lords in the British constitution. It is asked to approve the EEC budget each year (and can delay its consent), is consulted about issues bound for a decision by the council, can raise specific issues (complaints at the effect of Commission decisions on member states, for example) and can draw up and approve reports on broad policy questions (as on environmental issues) which, although not binding on the Commission, are regarded seriously in Brussels.

European federalists naturally look forward to a time when the influence of the European Parliament will have so grown that national parliaments have no more than the importance of state legislatures in the United States. Others, and governments such as the British, have a different view of the future.

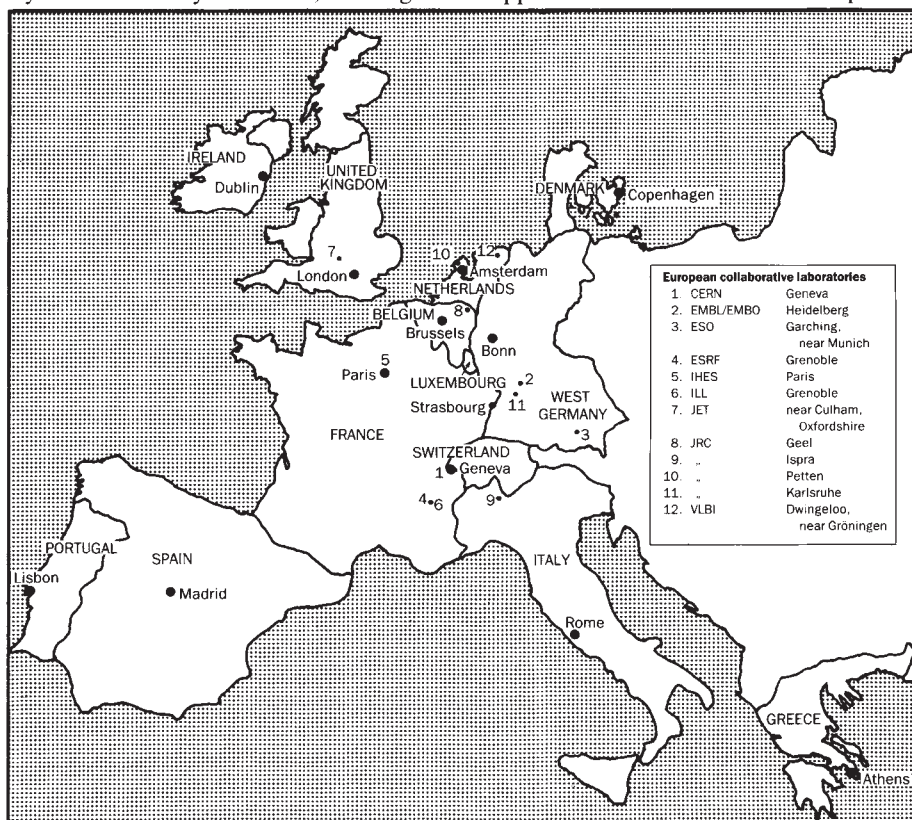
Constitutionally, it is a plain fact that the Treaty of Rome, even as amended by the European Single Act, does not compromise the sovereignty of national governments in the fields of defence, economic (as distinct from trade) policy and foreign affairs. Such forms of collaboration as there may be in these areas are voluntary. Similarly, the Commission's writ does not run to educational policy, either at school or university level, although there is a newly appointed 'task force', run by Welshman Hywel Jones, seeking to

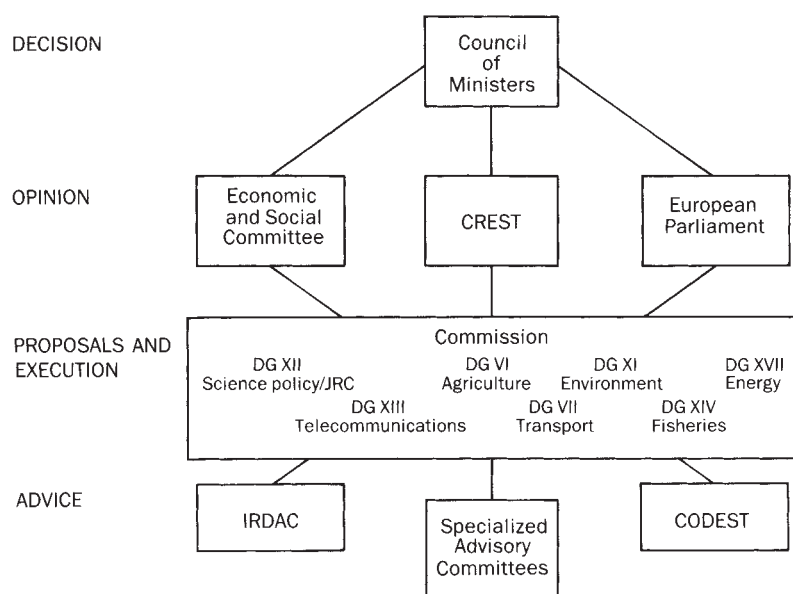
define educational issues to be tackled.

It is also relevant, not merely to the constitutional limits of the Commission's own powers but to its own independence, that commissioners are appointed (and may be removed at the end of their five-year terms) by national governments individually. In office, commissioners function corporately as if they were the cabinet of an administration, but are also given charge of specific portfolios (areas of responsibility); each runs one of the Commission's departments, headed by a director-general, who is in turn responsible for a section of the Commission's staff (most of whom are also seconded to Brussels by member governments).

The Commission's independent powers (comparable in many ways to the now-truncated power of the US administration to make war) stem from the provisions of the Treaty of Rome requiring that member states should compete with each other on equal terms. The undoubtedly proper exercise of these powers is nevertheless one of the reasons why the Commission has won a reputation for bumbledom: its directives (on matters such as the definition of particular food-stuffs), which have the force of law in all member states, seem to arouse most derision. Much of the Commission's regulation of environmental standards derives from the same power.

It seems not to have been remarked that, in principle, the Commission's powers to require equal competition would allow it to regulate government support for research and development





Commissions in context — the structure of the European bureaucracy.

directed towards the support of its domestic industries.

So far, the Commission appears rather to have concentrated on the development of its own research policy, which is at present a corporate issue spanning at least three of the directorates-general — those responsible for research, industry and environment. Of these, that responsible for research, known as DGXII (pronounced “Dee-Gee-Twelve”) plays a crucial role, both as a prime mover behind the five-year ‘Framework’ programme now half completed, and as the small and only source of support for basic research.

So how fair is the charge of “bureaucrat”? There is no simple answer. But the popular European view that Brussels is filled with a huge army of civil servants bent on interference is wide of the mark. The staff of 17,000 might be held to be small, given the need to administer such a variety of policies in a dozen countries speaking eight acknowledged languages.

The legalism of the Commission's procedures, especially in the drafting of its directives, is probably unavoidable given their force as trans-national legal instruments. The triviality of some of the issues dealt with (the definition of ice-cream, for example) probably reflects the influence of parochial lobbies (to which Brussels is prone), but might be avoided by clear-headed forward planning.

No doubt the directorates-general differ, but first impressions do not suggest that the senior people have a bureaucratic caste of mind. Many of them are Europeans impatient for more rapid progress towards a united Europe. Within a directorate, at least, there is also room for flexibility; DGXII, for example, is frequently able to make small grants of money to help organize meetings on issues that may lead to some collaboration. Nor do the Commission's dealings with the outside world fairly suggest that armies of

pernickety people have deliberately designed forms so complicated as to deter respondents from completing them. On the contrary, the Commission's more common failing is that too much of what it does is done with too much haste, often reflecting people's eagerness to see that money is spent as soon as the appropriate budget has been approved.

Yet there is a sense in which Brussels is a bureaucracy in the worst sense, perhaps unavoidably. The Commission as a whole does not have the flexibility, enjoyed by elected governments, to second-guess

IMITATIONS

Those things that the EEC is not

London

POST-WAR interests in cooperation have littered Europe with international organizations which are often confused with the EEC. Here is a brief guide to them:

- The Council of Europe is a strictly European organization meant to foster collaboration in the fields of law and social policy (which may include, for example, the regulation of research). There are now 26 members. Its chief functions are to negotiate international conventions, which have the force of law in member states; the best-known is the Convention on Human Rights, signed in 1950, accompanied by a European Court of Human Rights which hears complaints by individuals against their governments on the recommendation of the European Commission on Human Rights. The council's headquarters are in Strasbourg.

- The Organization for Economic Cooperation and Development or OECD began life as the Organization for European Economic Cooperation. The name was changed in 1961 with the accession of the United States and Canada and, at a later stage, Japan and Australia. Outside the

their electors, shifting resources in this direction or that as circumstances change. Line-item budgetary control is implicit in everything its political masters allow it to do. Worse, the commission's own procedures are not as coherent as they should be.

For such a young organization as the Commission (or perhaps precisely for that reason), the directorates-general are surprisingly jealous of each other's powers of decision. This is the mechanism by which the Commission appears frequently to reach nonsensical and corrosive decisions on questions such as the use of hormones in growing beef cattle; the power of decision lay with those responsible for agriculture and environment, who chose not to consult those in DGXII who would certainly have known better.

The question whether Brussels might arrange to seem less bureaucratic is a question of its own deportment. Of necessity, the mostly intelligent and frequently idealistic people working for the Commission differ from those working for national governments in their conviction that the destiny of Europe is to be united. They naturally regard the member governments as entities that must be helped, and sometimes hectored, to share the same vision; with the passage of 30 years, their signature of the Treaty of Rome seems to count for little in Brussels now. Could that be why there seems always to be at least one, and sometimes several, among the member governments which is or are uneasy with the process they began? □

field of economics, OECD has a section of science and technology best-known for its collections of statistics on scientific manpower and spending and for its occasional reviews of national science policies. OECD also operates the Nuclear Energy Agency and the International Energy Agency. The organization is based in Paris.

- The Western European Union is a treaty between seven governments, including Canada and the United States, which among other things is the forum in which the West German government has undertaken never to make nuclear weapons and in which Britain and the United States have undertaken to maintain armed forces in mainland Europe. The headquarters are in London.

- EUREKA is the organization set up on a French initiative in 1985 to arrange for European collaboration on the development of marketable products. Originally advocated as a mechanism for stimulating European industry because, at the time, it was suspected that US industry would be stimulated by the Strategic Defense Initiative (SDI), Eureka now has an administration based in Brussels □

Budget an endless bone of contention

Brussels

By the standards of many governments which are members of the EEC, the Commission has a substantial budget for research. In the five-year period ending with 1991, the Commission will have been spending roughly 1,500 million ECU a year, but the research people at Brussels are now excited by the prospect that their collective budget will be increased to 2,500 million ECU a year from about now.

Even at the present level of spending, the Commission's research budget is, in round numbers, virtually on a par with what Britain calls its Science Budget, spent through the research councils on university and in-house basic research. It is also roughly equivalent to the combined federal subventions in West Germany of the Max-Planck Gesellschaft and the Deutsche Forschungsgemeinschaft. But officials here insist that their budget is roughly 4 per cent only of total European spending on research.

The prospect that there will now be an increase seems to be a little-noticed consequence of the budget agreement in the closing days of 1988 at which member governments agreed that there should be a further squeeze on the cost of supporting European agriculture. As yet, there are no firm plans for spending the extra money. (There are a few suggestions elsewhere in this section of *Nature*.)

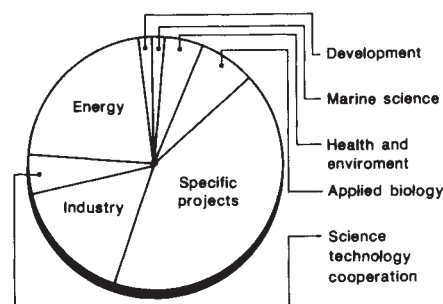
This is a far cry from the second half of 1986, when it seemed as if the British government would carry its opposition even to a reduced programme to extreme lengths. Earlier that year, the British and West German governments had objected both to the scale and the character of what the Commission calls its "framework" programme. Even after the West German position had been modified, the British government persisted with its opposition, acquiescing only after 1988 had begun.

Curiously, direct authority for the Commission's research programme derives only from the Single Act signed in February 1986, which leaves the Commission in no doubt of its responsibilities. The chief task is to strengthen European industry and to help make it more competitive. Roughly 42 per cent of spending in the current programme is under the heading of "Towards a large market", most of it in telecommunications, but 100 million ECU is set aside for traffic and travel projects. Energy (22 per cent), industrial modernization (16 per cent) and applied biology (5 per cent) consume a little more than that (see figure).

The general way of working in the industrial field is that the Commission offers research and development grants on a

50:50 matching basis to consortia of industrial companies or other organizations (such as universities and national government laboratories). A precondition of an award is that the partners must be drawn from at least two European countries. The best-known of these programmes is the telecommunications programme, ESPRIT, on which 2,350 million ECU will have been spent between 1986 and the closing months of 1992.

But the Commission also has research projects which are more particularly its own, outstanding among which is the controlled (and high-temperature) thermonuclear fusion programme whose chief embodiment is the JET (for Joint European Torus) thermonuclear tokamak device in Oxfordshire, England. This pro-



The Commission's current research spending. The programme will have cost the EEC more than 1,200 million ECU over six years by the time the present phase comes to an end in 1991. (The Commission meets 80 per cent of the total cost.) The design of the next machine, called NET (for Next European Torus) is under way but a decision on its construction (and even siting) has yet to be made. The Commission also contributes to the cost of some national thermonuclear programmes as at Garching (West Germany) and Frascati (Italy).

More contentiously under the Commission's own control are the laboratories grouped together under the name Joint Research Centre (see page 732), mostly inherited from the Euratom organization (which, like the European Iron and Steel Community, was one of the precursors of the EEC). There are four laboratories in Belgium (Geel), Italy (Ispra), the Netherlands (Petten) and West Germany (Karlsruhe), but the work is organized into nine divisions (called institutes), dealing with topics as different as transuranic elements (Karlsruhe), materials (Petten) and environmental safety (Ispra).

The centre's budget problems have their roots in EEC history. Euratom was conceived of as a research organization, but later acquired responsibility for the operation within Europe of safeguards on the use of fissile materials. When Eur-

atom's member states were full of enthusiasm for nuclear energy, it seemed natural that the organization should be ready to build prototype reactors of all kinds, for which purpose research stations were plainly needed. But then the gilt disappeared from that gingerbread.

Ispra, the largest site, has been consistently the most troublesome. Now, after successive painful reorganizations, the laboratory is on the way to being a kind of European Standards Laboratory with responsibilities, on the side, for nuclear reactor safety and environmental safety issues. But the Commission's continued support of the Joint Research Centre was one of the causes of trouble in 1986.

By the standards of these expenditures, basic research comes a poor last (page 734). Spending on the relevant part of what is called the EEC's stimulation programme has been fixed at 167 million ECU over the five years to the end of 1992 (up from a total of 60 million ECU in the previous three-year period). Transnational collaboration is a precondition for a successful application, but matching funds are not required.

On the face of things in Brussels, there is general contentment with this pattern of spending. The common goal is to create the single market and somehow to arrange that it is competitive with the rest of the world. If there is a research programme, and especially a substantial one, is it not self-evident that it should be pointed in the same direction?

To outsiders, that logic may not be nearly as compelling. If, for example, the objective of the creation of the single market is that competition between companies should be free and efficient, without reference to their geographical location, will it continue to make sense that most forms of EEC research support should be devised so as to marry efficient companies with less efficient collaborators? And if the Community is concerned about its supply of skilled manpower, might there not be something to be said for strengthening the infrastructure?

Asking such questions in Brussels does not physically endanger one's person; indeed, people are very polite and are willing to acknowledge that they are questions that should be asked. But of whom? The Commission has problems enough on its mind, the European Parliament is preoccupied with the environment and bothered by the discomforts the single market will bring to its constituents. The main council (which meets only twice a year) will have horrendous political disagreements, such as that over monetary union, to resolve by 1992, while the sub-council on research could hardly settle on a change of direction without approval from above. Yet it will be a pity for Europe's dreams to go unanswered because nobody has the time to listen to them. □

Sponsored research but with strings attached

Brussels

FOR the time being, the cornerstone of the Commission's effort in research is the conviction that European industry must be helped to be more competitive with the outside world, and that the sponsorship of industrial research is the best way of doing that. How well is it doing?

The best-known and the first major collaborative programme is that known as ESPRIT, intended when launched in 1984 as a ten-year programme of research and development in information technology, financed on a cost-sharing basis between the Commission and collaborating companies. (The Commission's share is more than 300 million ECU a year.)

ESPRIT inevitably became the model for later programmes. One of these, BRITE, is a cost-sharing programme aimed at improving the production technology and thus the competitiveness of small and medium-sized enterprises, SMEs in Eurospeak, on which the Commission has spent 175 million ECU on 230 projects since 1985.

The diversity of these programmes is widely remarked upon, as is the enthusiasm of those who take part. ESPRIT, for example, covers everything from the design of the semiconductor elements of computers to the development of software and hardware for desk-top publishing.

Out of the first of these interests has grown the programme called JESSI on the development of submicron computer chips (see *Nature* 337, 682; 23 February 1989). Garcia Aroyo, the DGXII director with responsibility for technological research, appears sanguine at the prospect that his colleagues will soon be labouring under 800–900 applications for funds to support industrial research in production technology and materials development. BRITE was merged with a parallel programme on materials development from the beginning of this year; the closing date for applications under the combined programme (on which the Commission will spend 500 million ECU in the next four years) is 12 May.

The rules are the same. Successful applications must involve industrial companies from more than one European country (and not more than two-thirds of the total grant can finish up in any one country). Companies must be genuinely independent of each other (which means that the collaboration cannot simply be a means by which one company and its subsidiaries elsewhere collaborate on work they might have done anyway). A small proportion of the funds (up to 7 per cent) may be spent on proposals by university consortia for generic investigations provi-

ded that the proposals are endorsed by industrial companies.

Projects must be original and preferably applicable in more than one industry. Interestingly, companies from countries in the European Free Trade Area (Norway, Sweden, Finland, Austria and Switzerland) may take part, but cannot receive Commission funds (and may have to contribute modestly to administrative costs). Research projects must be "pre-competitive". This time round, there is also a scheme for providing up to 75 per cent of the cost of a feasibility study (up to a maximum of 25,000 ECU).

This year's programme has plainly been much influenced by the report of an influential evaluation panel under Yves Farge, vice-president for research and development at the French company Pechiney et Cie., which reported last July. That panel, for example, recommended the merging of the BRITE and advanced materials (EURAM) programmes.

The Commission has also, in a late amendment of its proposals, followed the recommendation that universities collaborating in industrial projects should be recompensed by the Commission for the full cost of their work. (Under the old arrangements, industrial partners would usually pay the other half of the cost.)

Of the questions raised by the Farge committee, those implicitly answered so far are procedural in character. More fundamental issues are unresolved, conspicuously the panel's concern at what it called a "major contradiction" in the Commission's restriction of the programmes it would support to those judged "pre-competitive".

The principle is that the Commission will not support industrial development work leading directly to marketable products or usable processes, but only industrial research of a generic character on which more pointed research programmes can be based. The experience of the BRITE programme apparently showed that some "excellent projects" were not supported because they were judged to be "too competitive", but that the fear of falling into that trap pushed other applicants "upstream", with the consequence that the industrial interest of their projects was diminished. This complaint is also a dilemma for the Commission. Relaxing the pre-competitive rule would place the Commission in the position of providing direct support for commercial development by individual companies.

But the Farge panel points to the recent amendment of anti-trust legislation in the United States allowing research and development partnerships, and says that

Rooting for grants

SELF-EVALUATION is the price the Commission has to pay for its existence. Councils of ministers insist upon it. But one official describes the process as the administrative equivalent of repeatedly digging up a newly planted tree to see whether its roots are growing.

The Farge panel, for example, was in a position to attempt to estimate the economic benefits that would flow from the two first phases of the BRITE programme by means of a questionnaire to the industrial companies participating.

Among other things, participants were asked to estimate the degree to which the project on which they were working would increase their annual turnover, from which the Farge panel estimated that the total increase of annual production resulting from BRITE projects would exceed 2,000 million ECU. But the panel also noted that the same companies planned to commit only "modest" sums to the further development of the outcomes of their projects, for which reason it suggests that the economic benefits expected of the projects should "be viewed with some caution". □

"the law should be the servant of the Community, not its master". The panel also complains that the Commission, in its management of its research programmes, has not been sufficiently explicit about its objectives.

More generally, the panel pointed out that it had not been able to put the BRITE programme "into perspective in a coherent approach to an industrial research and development policy". This criticism appears only partly to have been met in the new phase of the programme.

BRITE covered nine disparate fields such as computer-aided design (CAD) and production technology for flexible materials (mostly textiles). Its successor has only five broad headings, which may be thought a step towards the definition of a strategy. Three are aeronautics, advanced materials and design, quality and reliability. There are also subprogrammes for the application of known manufacturing technology and the development of novel techniques and processes.

The Farge panel was also concerned that the Commission's own judgement of which projects to support is excessively technical, and insufficiently informed by business considerations. (The Commission's technical staff do get high marks for competence and enthusiasm.) The consequence, the panel said last July, is that there was too much "technology push" and not enough "market pull" in the BRITE programme.

In the nature of things at Brussels, it is too soon to know how the next evaluation panel will rate the achievements of the new programme. Much will depend on how the Commission's officials adapt their choices to the Farge's panel's pleas. The test will be whether Europe's middle-sized businesses are markedly more efficient. □

Linking an optical cable to every house

Brussels

WHO knew that Europe is building the hardware and software for a broad-band communications network that will begin to be a reality six years or less from now? The goal is to develop, by 1995, the technical ingredients required for a digital communications network capable of distributing through optical cables both high-definition video signals and huge quantities of data. Brussels seems confident that the project will succeed.

In many ways, the RACE project is what Pierre Aigrain, the French government's chief adviser on science and technology in the late 1960s, was then pleading for (to the EEC). But the new project's ambitions go far beyond Aigrain's hopes. One of the objectives, for example, is that all European dwellings, not simply business offices, should eventually be connected to the network. Meanwhile, there will be high-definition television for all, and pan-European mobile cellular telephony.

According to Roland Hüber, the director in charge of what is called Directorate F (a branch of DGXIII, the Commission's telecommunications ministry), RACE has now let 85 contracts to groups of companies and other organizations (on the Commission's usual 50:50 basis). More than 230 companies are involved, including all the major manufacturers of telecommunications equipment in Europe (together with AT&T and IBM). There are also 89 universities and other independent research organizations, as well as 11 PTTs. Collaborators from the European Free Trade Area are welcome.

As a means of organizing a major tech-

nical project, RACE is itself an innovation (which has been replicated in many of the Commission's projects). The pattern is determined by the circumstance that the Commission is merely the stimulator of innovation, not its eventual executor. Even so, the first step seems to have been to define objectives and to carry out a critical-path analysis for their attainment. But the objectives are not merely technical — the programme supposes RACE must also demonstrate useful applications of broadband networks, which means stimulating demand, and must then study some of the almost sociological problems that are bound to arise so as to demonstrate to eventual investors in the network that their money will be returned.

A further difficulty is that the Commission's funds cannot possibly be stretched to cover all the developments that will be needed for such a project, whence the decision that the Commission should advertise all the tasks that will have to be carried out, but to let contracts for the critical nodes and for a proportion of the others, leaving it to the competitive instincts of the disappointed applicants to pursue projects with their own resources.

The earlier phases of RACE (which began formally at the beginning of 1988) are largely technical, ranging from software development to the investigation of indium phosphide as an alternative to barium niobate in optical switching. Now, while the first crop of projects is under way, the Commission is looking for demonstrations of applications.

Even these phases of the programme offer everybody something. One of the

pilot application projects approved this year, for example, involves 15 European museums (the majority in West Germany) which have undertaken to develop means of displaying museum objects on workstation screens, together with associated sounds and images, so as to make museum collections generally available. The project requires the development of techniques for managing archives by means of expert systems. RACE is interested because similar techniques could be applicable in business. There are also two publishing projects — one involving the rapid transmission and processing of information that may be provided in different forms, the other more specifically concerned with the transmission and local production of newspapers.

A large part of the explanation for the change of climate is that the Commission can now boast of having won the collaboration of Europe's traditionally quarrelsome PTTs. One theme running through the report of a strategic audit of the project carried out by a team in which PTT managers were prominent is that national governments must quickly make up their minds about the regulatory framework within which the communications network will operate.

RACE is one of the EEC's more obscure acronyms (standing for R&D in Advanced Communications for Europe), but the word epitomizes the sense of urgency in its conception. The project owes its existence to the recognition in 1985 that the development of broad-band communications systems in Japan and the United States was ahead of that in Europe, and to a calculation that European companies and PTTs, left to their own devices, would not be able to bridge the gap.

By 1986, the Commission was asking for 800 million ECU to support its work-plan, but the request was trimmed to 550 million ECU in the framework budget eventually agreed. Now the Commission is asking that some of the funds should be restored, as it has emerged that many of the projects it has backed will need five, not three, years to complete.

The management of the programme sets great store by the biannual meetings at which all the project teams are represented. Otherwise, projects are autonomous and, as with other industrial research supported by the Commission, members of the project teams make their own arrangements for sharing patent rights and, in due course, such profits as may flow from their development. But the Commission has the right to require that patent rights should be licensed on reasonable terms to other European companies. Beginning in 1992, it is intended that there should be demonstrations of the kinds of integrated networks that may be built from the components that will by then have been developed, at

APPLIED BIOLOGY

Travelling a slow road to new technology

Brussels

THE Commission's research strategy gives applied biology a central place, but progress has been slower than people in Brussels were hoping as long ago as 1980, when the Commission began modestly to support biotechnology projects. Not that enthusiasm has dimmed, but regulatory hesitations by some member states are one cause of delay. Another is that the European blend of well-established pharmaceutical and chemical companies and small start-up companies does not easily lend itself to the kind of financing with which the Commission is most familiar.

The Commission nevertheless maintains a kind of cheerleading organization called the Concertation Unit for Biotechnology in Europe (or CUBE) which is a fertile source of ideas and, occasionally, of smallish pro-

grammes backed by Commission funds. One of its tasks is to steer Europe towards applications of biology that will not further increase present embarrassing overproduction of many kinds of foodstuffs, whence the strategy of encouraging the development of new kinds of crops having a greater nutritional value or containing important industrial raw materials. Using biotechnology in pest control is another goal, as is the development of techniques for using the whole of a crop plant — not just, as in the case of cereals, the seeds.

The name for the new programme, launched last year, is ECLAIR. The budget is a comparatively modest 72 million ECU over five years. But the programme sidesteps contentious questions about the release into the field of genetically engineered organisms. □

first on a laboratory scale, afterwards more generally.

In the end, the success of RACE will hang on the extent to which it influences the development of a broad-band communications network in Europe — and when. Much will depend on the speed and the vigour with which national governments are prepared not merely to enforce common technical standards but to create market conditions in which broad-band communications can prosper.

There are thus political as well as technical problems to solve, not the least of which is that of requiring European PTTs (most of which remain nationalized industries) to allow outside commercial organizations to lease communications channels in the broad-band network for

the provision of what are called value-added services — video signal transmission, for example.

The obvious danger is that different governments may choose to deregulate their PTTs in different ways, thus replacing the present system in which monopolies do what they wish within their territories to one in which national patterns are as different from each other as at present. The Commission spelled out the dangers in a paper published last year, but it has no power to turn its wishes into reality. RACE itself seems convinced that the pace of technical change will shorten its own timetable, but that may be an over-cheerful view. Political difficulties that remain unsurmounted could work the other way. □

RESEARCH FOR INDUSTRY

Cause and effect in a competitive world

Brussels

THE European Commission is outspoken about its policy on industrial research, and on research in general. Starting from the calculation that the twelve EEC members spend less on research and development than the United States, both in absolute terms and relative to gross domestic product (GDP), and less than Japan by the second yardstick, the Commission concludes that the objective of its research policy must be to make Europe "competitive", pursuing "where appropriate, its own technological options".

The figures, conveniently collected in the *First Report on the State of Science and Technology in Europe* presented to the European Parliament at the beginning of this year, are chastening.

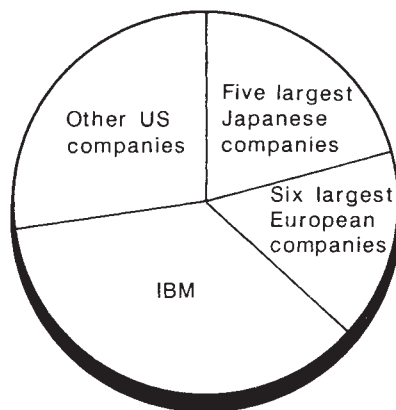
In 1985, EEC spending amounted to 76,250 million ECU, compared with 134,645 million ECU in the United States and 48,056 million in Japan. As a proportion of GDP, European spending was 1.9 per cent, compared with 2.8 per cent in the United States and 2.6 per cent in Japan. The same report notes that non-defence research and development expenditure in Japan is growing twice as quickly (9 per cent a year against 4 per cent a year) as in Europe and the United States.

The Commission is also disturbed by what seems to be a manpower imbalance. According to the US National Science Foundation, in 1986 there were 69 US workers in research and development for every 10,000 in the total labour force, compared with 63.2 per 10,000 in Japan (in 1985) and corresponding figures (in 1984) of 49.1 in West Germany, 41.2 in France and 32.8 in Britain. The report again notes that only the Japanese figure is growing quickly.

The figures for the output end of the

equation are equally sobering. The bulky annexes to the report record in detail that, in industrial sectors as different as chemical production and machine-tool manufacture, EEC performance is in relative decline.

The figure below shows the EEC's estimate of the world's data-processing



market in 1987 secured by the 20 largest companies. The report notes that sales outside Europe of even the largest of the European companies are a small proportion (15 to 20 per cent of their turnover).

The Commission's argument from this point on is simple. There is an imbalance between the EEC and Europe's principal competitors both in efforts in research and development, and in industrial performance. So is it not reasonable to link the two as cause and effect?

The conclusion is that the three goals for EEC research are to improve Europe's international competitiveness, to "increase its capacity to pursue its own scientific and technological options, where necessary by reducing its dependence on others" and "to respond to the needs of society by

improving the quality of life".

The Commission concludes that there are five areas in which European efforts should be concentrated, as follows: information technology and telecommunications, industrial materials, aeronautics "where Europe faces a particularly important competitive challenge", the biological sciences (including biotechnology) and energy.

It is clear that aeronautics will feature prominently in the Commission's next Framework programme. The argument is largely economic, but the competition is exclusively from the United States. The volume of civil air transport is growing at 7 per cent a year, and roughly 40 per cent of operating costs are determined by the cost of the aircraft, while there is also a military market in which, the Commission said in a document published in June last year, US restrictions on the sale of advanced aircraft are worrying.

The plan is that a pilot programme of coordinated research will be drawn up later this year with the intention that there should be a full-blown research programme looking for funds in 1990. For as things are, the Commission said last year, "European industry remains largely divided between national interests. . . . Continuation along this path can only lead to failure".

This important document also deals with the balance between basic and applied research, noting at one point that many European governments in recent years have effected a redistribution of resources from basic to applied research and adding "there is a risk that the pendulum will swing too far . . . just [when] our competitors are placing a greater emphasis on basic research". This is said to be a reference to the growth of the US basic research budget during the Reagan administration and to recent calls in Japan for more attention to the same cause.

On the related issue of the discrepancies within the EEC between the performances of different governments, the report notes that spending on research and development in West Germany, France and Britain (in that order) accounts for 75 per cent of all EEC spending, and that the proportion of GDP spent on research and development ranges from a maximum of 2.8 per cent in West Germany to 1.5 per cent in Italy, 0.8 per cent in Ireland and less than half of that proportion in Greece.

The discrepancy is described even more vividly in an annexe to the report prepared by the Irish National Board for Science and Technology, which calculates that the GDP per head of the four poorest countries in the EEC (Ireland, Spain, Portugal and Greece) have an average GDP per head less than 70 per cent of the Community average, but spend (per head) only 25 per cent as much on research and development. □

A new vision of unity in standards and training

London

JEAN-PIERRE Contzen, the Belgian director-general of the Commission's Joint Research Centre (JRC), has a vision in which the nine research institutes under his wing will become an amalgam of a standard-setting organization and a means of training Europeans in new technology. Arguing that Europe has too few public research laboratories, his belief that the JRC has a future is infectious. But the future has not always been as certain.

In reality, JRC has only in the past five months emerged from the most serious threat to its existence — the plain discontent of several member states most openly expressed during the long haggle over the present Framework programme. The complaints were that the four laboratories (then distinct) were too costly, too large, badly managed and without function.

The outcome was a review process carried out by a panel under Dr Harry Becker, research director of the Royal Dutch Shell Oil Company, which began in July 1986, and whose chief recommendations were structural and managerial in character. Crucial among these was the decision that the research programme and the administration of JRC should be overseen by an independent board, whose first chairman is Sir John Kendrew, the British Nobel prizewinner.

Commitology

BRUSSELS is as prolific in the invention of committees as in that of acronyms, and even uses the word *commitology* to describe the theory and practice of committee creation — do committee members represent member states? Who nominates them? Which arm of the EEC reimburses members for travelling expenses? Are decisions by consensus? If a decision is reached by a simple majority, can the chairman break a tie?

This year, each of the nine institutes of the Joint Research Centre (this page) will be blessed with an advisory committee. Most of the Commission's research programmes have one of their own as well.

But in the Commission's administration of science and technology, the most powerful committees are CODEST (page 734), widely respected for its independence; CREST (representing national governments and advising on general strategy), commonly said to be too big to be workable, let alone manageable; and IRDAC (which stands for "Industrial Research and Development Committee"), which suffers from the same faults. □

Contzen, previously DGXII's planner (and thus the architect of the present Framework programme), is in the unusual position of having helped to review the organization he must now administer. Plainly there is some frustration that the legal procedures were completed only in November 1988 (the new plans had to be approved not merely by the Commission and the council, but by the parliament as well). But now, with relief, he says that the new organization is in place.

The most obvious change is verbal: the Commission used to operate four Joint Research Centres, at Karlsruhe (West Germany), Geel (Belgium), Patten (the Netherlands) and Ispra (in the foothills of the Italian Alps), but now there is just the Joint Research Centre, in the singular. The three northern laboratories have been renamed as institutes (but Petten, now called the Institute for Advanced Materials, has a kind of outstation at Ispra); as yet, their research programmes are little changed.

Ispra, always the worry, has seen the most upheaval. Its research programme has been divided into five parts, each of which is renamed as a separate institute. Ispra also becomes the location of the unit intended to provide the rest of the organization with research support, principally in computing.

Part of Contzen's immediate problem is psychological: how to give the 2,180 members of his staff the ideal blend of institutional loyalty and corporate 'solidarity'? The calculation is that the identification of separate institutes dealing with different parts of the research programme is a necessary means of making the separate functions pointed and of making sure that people know what they are about. But there is also a long-term need that people should move more freely than in the past from one programme to another.

Inflexibility is the most obvious difficulty. The Community's procedures are an encumbrance: line-by-line approval and scrutiny of the budget is routine, which means that there is little scope for transferring resources from one project to another as new opportunities arise.

The immobility of the staff is another impediment. Only about 3 per cent move to posts outside JRC each year. What Contzen hopes is that the future pattern of employment may change that. New appointments are now routinely made on a five-year contract, which may be renewed for a second five-year period, but not thereafter; either the person concerned is then appointed for an indefinite period, or he or she must leave.

More than half (53 per cent) are already

employed on short-term contracts such as these. It is now also planned that there should be a system of three-year appointments that will be strictly non-renewable. The first ten posts have been advertised, but Contzen hopes there will be 150 of them in four years. He regards them not merely as a means of recruiting specialists for urgent tasks, but also as a kind of industrial postdoctoral fellowship that may be an innovation in its own right.

JRC also plans to foster its more familiar fellowship schemes (there were 11 graduate students and 24 postdoctoral fellows on the books last year) and to attract more senior people from elsewhere for periods of up to a year. (There were 58 of these sabbatical fellows in 1988.)

Contzen has undertaken that in the next four years, the annual turnover of staff at JRC will increase from 3 per cent to between 5 and 10 per cent a year. A plan to terminate the appointments of 100 staff members will help, but is bound to be a bone of contention in the council of ministers, which has been severely critical of the generosity of previous early retirement schemes.

Salaries at JRC (and at other Commission establishments, such as JET) have always been a contentious issue, partly because they must of necessity be greater than those paid in the EEC's most prosperous member states, partly because they are tax-free. The other side of that coin is that the language and cultural barriers within Europe are still so great that the education of JRC employees' children is likely to be interrupted, as will be their spouses' careers.

The quota system is another stifling influence on JRC. Broadly speaking, the nationality of the professionals on JRC's staff must reflect the size of member states. It is not so much that governments have been anxious to secure for their own nationals posts in a well-paying organization (although there is some of that); others are or have been alarmed that JRC might suck away important parts of their skilled labour force.

The Commission (which is ambivalent on the issue) offers in defence of the system the reflection that it helps to diffuse JRC's work through the Community. But quotas are an irksome impediment to good management; Contzen wishes they would go away. The question of whether there would be quotas (and on what basis) in a united Europe seems not to have been considered.

Despite the impediments, the pattern of research is likely to change more quickly now than in the past. One administrative innovation is the decision that roughly 5 per cent of JRC's resources should be spent on the exploration of new projects.

Under this rubric, in 1988 JRC embarked on investigations of the use of ultrasound (21 kHz) for removing aero-

THE nine new institutes of JRC created by last year's reorganization are as follows:

■ **Institute for Transuranium Elements**, at Karlsruhe (West Germany), may be best known for basic studies of the physical and chemical properties of the actinides, but is also the originator of the project to use ultrasound for removing aerosols.

■ **Central Bureau for Nuclear Measurements**, at Geel (Belgium), has a reputation for precise measurements, most recently of the low energy fission cross-section of ^{235}U , but is also making precise measurements of the silicon lattice as part of the international redefinition of Avogadro's number.

■ **Institute for Advanced Materials**, at Petten (the Netherlands), is making a transition from the exclusive study of materials under neutron irradiation (for which it was originally established) to the development of new materials and the modification of their properties by surface and other engineering.

■ **Institute for Systems Engineering**, at Ispra (Italy), develops new safeguards techniques for nuclear inspectors, gathers data relating to the safety of thermonuclear fusion reactors but is well-known for a computer program for the assessment of solar input to groups of buildings as part of a general interest in solar heating and conversion.

■ **Institute of Safety Technology**, at Ispra (Italy), is building the reaction wall for the assessment of built structure under mechanical impulsion, but is still concerned with aspects of reactor safety including the study of cooling systems in experimental rigs. It has organized a study of the problems of handling tritium in bulk as will be required in thermonuclear fusion reactors.

■ **Environment Institute**, at Ispra (Italy), traditionally concerned with air and water pollution, has now commissioned a laboratory for the analysis of foodstuffs and drugs.

■ **Institute for Remote Sensing Applications**, at Ispra (Italy), mostly working on the design of novel instruments, has recently proposed to the European Space Agency a scheme for radar sensing of the Earth's surface and plans to use Lidar for the monitoring of fluorine compounds in the atmosphere.

■ **Institute for Prospective Technological Studies**, at Ispra (Italy), is in the process of being organized as a means of contributing to the analysis and formulation of DGXII's policy.

■ **Centre for Information Technologies and Electronics**, at Ispra (Italy), is one name for JRC's computer centre, but also has responsibility for the development of software and will arrange for the design and production of specialized chips. □

sols from large masses of air (which may be useful in dealing with chemical accidents), the applications of neural networks in computing and the further development of a non-destructive monitoring instrument for assessing plutonium in radioactive wastes. High-temperature superconductivity also featured in the programme last year as, no doubt, cold fusion will in 1989.

Broader considerations point to more radical changes in the years ahead. There is, for example, an obvious difficulty at Petten, the chief site of the Institute of Advanced Materials, which also houses JRC's High Flux Reactor originally built as a materials testing tool for Euratom. The reactor is now used for the routine testing of fuel rods of different design as well as for constructional materials used in reactors, but its future is evidently linked to the uncertain future of nuclear power. Accordingly, the operating power and the duty cycle of the reactor have been degraded, if only to prolong its life.

Another strategic consideration is that JRC will be safer if it can work more directly on projects relevant to the Commission's own needs. Data-collection and dissemination (on subjects as different as world shipbuilding and the properties of potentially hazardous chemicals) is an obvious opportunity. So is work on

environmental problems, where Ispra's Institute of the Environment obviously has an advantage.

There is a programme to use satellite observation data (from the French SPOT instruments) to compile agricultural statistics, using five chosen plots of ground in different parts of Europe. Ispra is similarly working on the improvement of instruments for the non-destructive measurement of fissile materials as used by the inspectors who operate nuclear safeguards procedures. (The European Community has secured an undertaking from the International Atomic Energy Agency that its member states' compliance with the Nuclear Non-Proliferation Treaty will be assured by the reports of its own inspectors.)

In the same spirit, the research group at Ispra originally concerned with the safety of nuclear reactors, and now renamed the Institute for Safety Technology, has spread its wings also to cover the safety of civil engineering structures and of industrial plants. The group should have finished next month the design of a structure (called a reaction wall) for the measurement of seismic and other mechanical impulses to structures. It has also become a source of expertise in the application of finite-element techniques in the design of engineering structures.

But much else of JRC's work is of a general character, relevant to the Commission's needs only in the most general sense. Conversely, there are fields in which the Commission would not at present turn to JRC for advice on the solution of all its problems — the use of hormones in growing beef cattle, for example, despite Ispra's interest in NMR techniques in the analysis of foodstuffs (the alcoholic content of wine in particular; see the News and Views article on page 708 of this issue) or the development of technical standards in telecommunications (where other organizations are more expert).

Yet the new research commissioner, Filippo Maria Pandolfi, has been saying since he took office at the beginning of this year that he wants DGXII to concentrate more of its resources on 'pre-normative research', which is eurospeak for work that may help to strengthen the Commission's administrative hand, from the standardization of equipment (as in telecommunications) to the basis for environmental standards and even the promulgation of standards used in trade and commerce, the work of old-fashioned standards laboratories for example.

In a broad sense, that is also a central objective for Contzen — but nobody wants JRC to become a kind of International Bureau of Standards for Europe.

Budget pressures will also shape the future pattern of JRC. The Commission has decreed and the board has agreed that JRC should find a greater proportion of its income from outside the Commission's budget, and indeed the annual report for 1988 (issued earlier this month) says that contracts already signed will contribute about 30 per cent to JRC's costs during the four years ending in 1991. But that calculation is something of a fudge.

The external contributions (over four years) include 55 million ECU for the operation of the Petten reactor and 59 million ECU for the support of Commission activity in agriculture, the environment and the like. Genuine external contracts now signed will bring in only just under 20 million ECU by 1991, although JRC's reorganization (and the recognition that its future depends on selling its services) will no doubt increase that amount substantially. But operating costs are less than half the total: 'personnel credits' amount to 132 million ECU out of this year's total budget of 239.4 million ECU.

Indeed, Contzen is also quick to argue that these calculations do not give JRC credit for the work it does as a partner in several of the consortia contracted to work for the Commission's industrial development programme. (The rules prevent programmes such as RACE from paying money to JRC.) Institutes collaborate out of interest, but there is a possibility that royalty income will eventually accrue. □

Larger budget still too small for comfort

Brussels

THE Commission's programme for the support of basic research has a new budget (33 million ECU a year until the end of 1991) and also a new name — SCIENCE — which is a triumph for whichever directorate-general looks after the invention of Community acronyms. But the budget, some 3 per cent of the Framework programme, is too small for comfort — and in 1988 the SCIENCE programme made 114 grants worth a total of 36.8 million of ECU.

Not that those who administer the programme are ungrateful for last year's increase, which has virtually doubled the funds available each year, and which represents a very substantial increase over the 6 million ECU available in 1984, in the first year of what was then called the Stimulation Programme. (No less than 1.5 million ECU was then spent on a project in which half a dozen universities planned to collaborate on optical computing.)

The SCIENCE programme is the nearest thing to a grant-making research foundation that there is within the Commission's organization. Its chief objective is to encourage collaboration between basic research groups, almost always by means of grants that supplement the funds available from national sources. The same budget has been used on three recent occasions to make grants to the European Science Foundation to support networks of European researchers.

Applicants for funds should be wary. SCIENCE does not pay for expensive pieces of equipment, nor does it provide running costs while, like all grant-making organizations with small budgets, it is torn between the wish that the projects that it backs will prosper and the fear that, if they do, they will wish to turn themselves into permanent pensioners. Even so, the programme is ready with examples of projects which have gone well — sometimes, well enough to be partners in larger Commission projects under other programmes, the BRITE programme for example.

One of these is the research programme on new permanent magnets typified by the NdFeB alloys which was begun by the Stimulation Programme in 1985 and which now embraces 120 people from 58 different laboratories (most of them, interestingly, at universities). The collaboration is following up the discovery in 1963, by the Sumitomo Special Metals Company and General Motors, that sintered materials with the approximate composition $\text{Nd}_{17}\text{Fe}_{77}\text{B}_6$ make permanent magnets of high field strength and coercivity.

The project has the virtue of being interdisciplinary as well as international

— there have been problems of growing single crystals, the determination of their crystal structure (which appears to be layered much as are the high- T_c superconductors), unravelling the relationship between magnetic properties and microcrystalline structure and fabrication techniques. (One remarkable development is exploitation on an industrial scale of the laboratory curiosity, called decrepitation, in which gaseous hydrogen interacts with solid $\text{Nd}_{17}\text{Fe}_{77}\text{B}_6$ newly cast from the melt to reconvert it into a powder.)

The collaboration's biggest prize so far seems to have been the discovery that the partial substitution of Nd by the rare earth element dysprosium (Dy) both increases the coercivity of the alloys and the temperature (below the Curie temperature) at which the exceptional magnetic properties are retained — important because of the temperatures likely to be encountered in the electrical machines incorporating these materials. The project has now won the accolade of a Euroacronym all of its own (CEAM) and support from the programme on advanced materials.

Last year, with more money to spend, CODEST appears to have backed as diverse a range of projects as anybody could wish. The handful of mathematics

SCIENCE PARLIAMENT

Voice for research in Brussels

Brussels

DGXII, which unlike most of the other directorates-general at Brussels is not much molested by lobbyists, has a scheme to mend its sense of isolation from its true constituents, the research community. Almost subversively, but keeping well within the rules, it plans to establish an assembly of working scientists which will represent the research community in Brussels.

The scheme is entirely within the gift of the Commission, which is free to decide how to spend the resources set aside for meetings of people. About now, the Commission should have taken the plunge. But some member governments are uneasy, suspecting that DGXII is looking for a means of recruiting scientific advice that will be an alternative to that provided by its own placemen on the innumerable Brussels committees.

The uneasiness may be well placed. The European Assembly in Science and Technology will consist of 200 people, a third of whom will be nominated by each of CODEST (see above), the Commission's basic research committee, the European Science Foundation (ESF, see page 723)

and computer science projects, for example, includes a three-year collaboration between the universities of Aberdeen and Paris-Sud to develop software that, starting from an incomplete theory and a mass of empirical information, will seek more complete laws and define the exceptions to them.

Last year's biggest projects include a 15-laboratory collaboration to make and characterize the structure of single crystals of high- T_c superconductors, which will cost the SCIENCE programme 1.36 million ECU over two years. Another project (involving laboratories in Belgium and the Netherlands with two in France) aims to build a laser capable of generating light intensities of up to $10^{20} \text{ W cm}^{-2}$ in 30 femto-second pulses, with the objective of following the multiphoton ionization of atoms produced (2.06 million ECU over 3 years).

Nineteen laboratories (nearly half of them British) are planning to collaborate on a basic study of the properties of magnetic recording materials with the deliberate intention of providing support for European industry (1.88 million ECU over 3 years), while the Netherlands Cancer Research Institute, the Pasteur Institute in Paris and the British Medical Research Council's Radiobiology Unit at Chilton, Oxfordshire, plan to pool their expertise in the genetics of the mouse to make a start on mapping the mouse genome (1.27 million ECU over 3 years).

Last year's largest collaboration, in

and national governments (*pro rata* by some rule).

The routine function of the assembly will be that of a panel of referees to whom all kinds of questions may be referred. But DGXII seems genuinely to be looking for new ideas. No doubt there will be opportunities for groups within the assembly to form committees to advocate what seem promising courses of action. There are also plans that the members of the assembly should meet together occasionally. Dreams that the assembly may be the world's first parliament of science are not far beneath the surface.

The practical difficulty may be whether 200 people can adequately represent European science and technology. The nominations not yet made will evidently be crucial. DGXII seems anxious that its assembly should not be mistaken for the European Academy, the feasibility of whose formation is being explored by Sir Arnold Burgen, a past foreign secretary of the Royal Society of London, and with ESF itself, a consortium of national grant-making organizations which has nevertheless sometimes mistaken itself for a European academy. □

which more than 100 researchers from eight Community countries are involved, is the study of plankton in the Antarctic seas organized by the Alfred Wegener Institute at Bremerhaven, in West Germany. Material was collected from the research vessel *Polarstern* during the Antarctic summer just completed, but its analysis will take a further two years (to which the Commission will contribute 551,000 ECU). And six marine science laboratories plan to study Quaternary sedimentation in the North Sea by means of a series of boreholes and with the help of a 1.51 million ECU grant over two years.

Among the grants CODEST did not make last year is that to which *Nature* has given some publicity — the proposal by the European VLBI consortium of radio-astronomers that the Commission should finance its plan for the re-equipment of the collaborating observatories and also support the proposed centre for the analysis of VLBI data at a cost of 17.8 million ECU.

In reality, the VLBI proposal would have consumed the whole of the SCIENCE budget for a year. The issue that remains to be decided is not whether the SCIENCE programme must be enlarged, but whether the Commission should devise a new mechanism for meeting such requests, which is subsidiary to the question of what the Commission's role in basic research is judged to be.

With all that said, in its small way, SCIENCE seems to enjoy a degree of flexibility not common in the Commission's works. Being small no doubt helps, but so does the advisory committee called CODEST which ultimately decides which projects shall succeed. (First, there is a refereeing procedure.) As if in recompense for its time and trouble in cutting up the small cake, CODEST has the right to be consulted about all proposals for developments in science and technology making their way to the Commission.

The chairman of CODEST since 1987 is Sir Peter Swinnerton-Dyer, chairman of the British University Grants Committee until that was transmogrified into the Universities Funding Council, of which he is now the chief executive. Unlike most of the Commission's advisory committees, CODEST members are not representatives of their governments but independent persons, which also gives the committee more legitimacy than that attaching to groups of government nominees.

Given the influence of CODEST in the Commission's scheme of things, as well as its independence, it is probably the institution in the EEC best placed to raise the important question of what the balance between basic and applied research should be. There is not much time left for tackling the question: the next Framework programme will be upon us before we know. □

Where to go next?

Brussels

ONE key figure in shaping the future pattern of the Commission's support of research and development is Dr Paolo Fasella, the Italian who has been director-general of DGXII for long enough to command the respect of a substantial part of European science. He is emphatically not a bureaucrat, but an enthusiast.

Last month, Fasella was basking in the outcome of the council meeting on 14 March. The decisions taken then bring to 98 per cent the proportion of the current Framework programme budget which has been approved (but some projects still have to work their way through the parliament). There is now a sense of expectation as people ask themselves what will follow. Will the expected increase of the budget from 1,500 million to 2,500 million ECU by the end of 1991 materialize?

The same meeting of the research council approved one of Fasella's favourite projects — that of using Community funds (30 million ECU by the end of 1991) to make better use of major installations scattered through the Community. The starting-point is the calculation that pieces of equipment as different as radiotelescopes and synchrotron light sources may be under-used because those for whom they were built do not, or no longer, use them fully. A synchrotron, for example, may have been equipped with fewer outlets on its circumference than can be accommodated.

Under the new scheme, operators of large EEC installations may be given grants with which to upgrade them, on condition that the equipment is then made available to researchers elsewhere in the EEC or, at first, elsewhere in Europe. Support will be provided for periods of three to five years. DGXII's hope is that it will soon have some 30 installations on its books.

On the more distant future, Fasella believes that the programmes of industrial research support will continue, even multiply, but that the Commission will find itself paying more attention to the needs of basic research. He says that many of DGXII's research activities, not simply its occasional grants to the European Science Foundation, help to form durable networks of people, which could usefully be multiplied.

H. J. Allgeier, director of the division responsible for planning and evaluation in DGXII and a strong advocate of the nascent aeronautics programme, goes further, acknowledging that it may not be long before the Commission has to face up to the responsibility of "standing behind" research institutions such as CERN and the European Space Agency (ESA). But at this stage, nobody seems willing to go to

the stake for turning the Commission's research programme into EEC's public grant-making agency.

It is not easy to see how this question will get the hearing within EEC that its importance demands. Most member governments are worried about their own economic performance in relation to that of other member states and also share the general view that competing with the United States or Japan (or both) is what EEC is for. They take the simple view that backing industrial research makes sense, but at the same time value the ability (or illusion) of seeming to shape national policy which national grant-making agencies can provide.

It is also the case that the Commission is not constitutionally well-suited to the role of a grant-making agency dedicated to the support of basic research and the pursuit of excellence. There would be obvious political difficulties if the research grants awarded by the Commission's hypothetical grant-making agency were not *pro rata* with member states' estimates of their national importance (by the yardstick of population, GDP or whatever). More seriously, most well-judged research grants would be discriminatory, not involving transnational collaboration, which the Commission is meant to foster.

There are nevertheless two directions in which the Commission could usefully feel out the ground ahead. First, it is anomalous that the Commission has so little direct concern with skill training and higher education; direct support of basic research might be a useful entrée.

Second, it would be entirely consistent with the Commission's present function that it should play a direct part in supporting collaborative research institutions, CERN, ESA and the smaller fry. Member governments would suspect that to be a way of spending their money by proxy and with less attention than they would exercise themselves, but there are many formulae by which the obvious dangers could be avoided. Why not make a few experiments? The VLBI proposal (page 720) would be a natural starting-point.

Commission support for basic research in member states might also be held germane to the Commission's function if it were regarded as one of the means by which economic imbalances within EEC might be in the long run corrected. There is already a Social Fund providing palliative cash. Why not research funds as well? Given that less prosperous member states' ability and readiness to spend money on basic research will always be deficient, the Commission's intervention may be the only sure way of increasing the general level of research spending in the Community. □

Unprepared for the single market

Will it be different after 1992? The general expectation that everything in Europe will change when the EEC (at last) becomes a common market is belied by the inadequacy of the preparation for that event.

THE EEC's research programme, the subject of the last part of this survey of European science, has always been controversial and will long remain so. The Treaty of Rome omitted to give the European Commission the power to sponsor research, because in the simple customs union then foreseen, the Commission needed merely the power to create the level playing-field on which companies in member states could compete on equal terms. In the 1950s, before research became a self-conscious component of public policy and long before governments recognized the link between research and prosperity, research belonged with cultural policy squarely within the competence of member states. Both the Iron and Steel Community and Euratom (which predated EEC) had research remits, but they had technical needs to satisfy.

Since then, the Commission has stumbled into research by historical accident — the coincidence of its administrative takeover of the two subsidiary communities with the rise of European anxieties in the 1970s about the 'technology gap' between Europe and the rest of the industrialized world. The Commission has responded manfully, but not always wisely, to this challenge. Its programmes described in the preceding pages are merely the most conspicuous. Yet now, with the prospect of a single market going beyond a mere customs union, there can be no doubt that the Commission has a proper role in research. The Single Act that promises great things for 1992 acknowledges as much. The danger now is that what it does will be the extrapolation into the future of present historical accidents. Several questions arise.

First, there is the matter of administration. In part, the Commission has been unfairly pilloried. The Joint Research Centre (once named in the plural) was in a bad way when the Commission took it over. Now, at least, there is a workable structure and a plan that may succeed (page 732). It should be given a fair chance. The more expensive cost-sharing programmes (page 729) are a more mixed bag. Much of the work they support is valuable — the evaluation panels say so — but they would be more valuable if they were not so often hastily thrown together. Often the Commission will spend well over a year winning approval for some scheme, give client companies and academics a few months in which to apply — and then spend more than a year making legal arrangements for the funds to be released.

Second, there is the matter of what the Commission's research is for. As stated, the purpose is to enhance industrial "competitiveness" while supporting only "precompetitive" industrial research. The Farge panel last year spotted that contradiction. Another, certain to seem sharper after 1992, is whether it will make sense to

insist that industrial research projects qualifying for assistance should always entail transnational collaboration when European competitiveness (with the outside world) could be based on a single efficient company. A further difficulty is that these projects seem to concentrate on innovation (often the 'me-too' kind) rather than efficiency, suggesting that self-sufficiency is the real goal.

Third, there is the matter of the balance between basic and applied research. The Commission has so far concentrated on the second, but acknowledges that Europe's future hangs not only on a torrent of innovation but on a sufficient supply of innovative people. The logic of that is that it should be spending a greater proportion of its funds on basic research, which is the only proven way of systematically turning talented people into innovators. The SCIENCE programme (page 734) is fine, but should be increased and also supplemented by a means of backing long-term institutional endeavours. The planned, but frustrated, VLBI scheme (page 719) is a test-case.

Fourth, there is the matter of the Commission's own dependence on science. Even among its constituents, the Commission has a bad name for making technical decisions in apparent ignorance of the ins and outs of technical questions. The decision last year to ban the use of anabolic steroids in growing beef followed the over-stringent standards for radioactive materials in food adopted in the aftermath of Chernobyl two years ago. But the Commission corporately is not nearly as ignorant as it makes itself seem. It is merely that when technicalities contradict what seem to be political, social or economic imperatives, it is easier to make a decision by neglecting to ask for the inconvenient advice. That may keep member-governments happy, but it is not a way to govern.

Finally, there is the related matter of the Commission's own personnel, researchers on its payroll, for example. As things are, the Commission is compelled by its member governments to operate a quota system: governments do not want to stuff ECUs into other nationals' pockets, especially in such large amounts. Will petty chauvinism of that kind make sense after 1992? And if not, how else are talented people to be employed? The question is again administrative, but it also goes deeper: the Commission expects 1992 to be a milestone on the way to a United States of Europe. If it had its time again, the Commission would no doubt start from somewhere other than the Treaty of Rome, perhaps from a common understanding on external relations. But history does not repeat itself for the benefit of those whom it inconveniences. The Commission's flair for dealing with its officials as if they were European could be another acid test of the validity of its ambitions for Europe. □

Observation of cold nuclear fusion in condensed matter

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When a current is passed through palladium or titanium electrodes immersed in an electrolyte of deuterated water and various metal salts, a small but significant flux of neutrons is detected. Fusion of deuterons within the metal lattice may be the explanation.

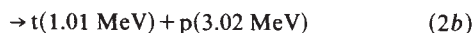
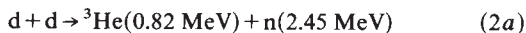
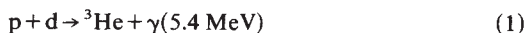
FUSION of the nuclei of isotopes of hydrogen is the principal means of energy production in the high-temperature interiors of stars. In relatively cold terrestrial conditions, the nuclei are surrounded by electrons and can approach one another no more closely than is allowed by the molecular Coulomb barrier. The rate of nuclear fusion in molecular hydrogen is then governed by quantum-mechanical tunnelling through that barrier, or equivalently, the probability of finding the two nuclei at zero separation. In a deuterium molecule, where the equilibrium separation between deuterons (d) is 0.74 Å, the d-d fusion rate is exceedingly slow, about 10^{-74} per D_2 molecule per second¹.

By replacing the electron in a hydrogen molecular ion with a more massive charged particle, the fusion rate is greatly increased. In muon-catalysed fusion, the internuclear separation is reduced by a factor of ~ 200 (the ratio of the muon to electron mass), and the nuclear fusion rate correspondingly increases by about eighty orders of magnitude. Muon-catalysed fusion has been shown to be an effective means of rapidly inducing fusion reactions in low-temperature mixtures of hydrogen isotopes^{2,3}.

A hypothetical quasi-particle a few times as massive as the electron would increase the cold fusion rate to readily measurable levels of $\sim 10^{-20}$ fusions per d-d molecule per second¹. The results reported here imply that a comparable distortion of the internuclear wavefunction can be realized when hydrogen isotope nuclei are loaded into metals under certain conditions. We have discovered a means of inducing nuclear fusion without the use of either high temperatures or radioactive muons.

Indirect evidence

Observations of naturally occurring ^3He in the Earth suggested to us new directions for laboratory investigations of nuclear fusion in condensed matter. ^3He is produced by the following fusion reactions:



Tritium (t) decays with a 12.4-yr half-life to produce ^3He . The well established high $^3\text{He}/^4\text{He}$ ratio in solids, liquids and gases associated with volcanoes and other areas of high heat flow⁴⁻⁶ suggests fusion as a possible source for the ^3He .

To estimate a possible rate of fusion in the Earth, we assume a simple, steady-state model in which the known flux of ^3He out of the mantle, 2×10^{19} ^3He atoms per second⁷, arises from p-d fusion occurring uniformly in the mantle water reservoir, taken as $\sim 1.4 \times 10^{24}$ g (R. Poreda, personal communication). Note that if the Earth contains 'primordial' ^3He , our calculated

rate will be an upper limit; on the other hand, if fusion-produced ^3He is stored in the mantle (so that the outward flux does not equal the production rate), our value will be a lower limit. As each p-d fusion produces one ^3He atom, and as the isotopic abundance of deuterium in water is $\sim 1.5 \times 10^{-4}$ deuterons per proton, we infer a geological fusion rate constant, λ_f , of

$$\lambda_f \approx \frac{2 \times 10^{19} \text{ } ^3\text{He atoms s}^{-1}}{1.4 \times 10^{43} \text{ deuterons}} \\ \approx 10^{-24} \text{ fusions d}^{-1} \text{ s}^{-1} \quad (3)$$

This rate is fifty orders of magnitude larger than that expected in an isolated HD molecule, and fusion at this rate could be detected if reproduced in the laboratory.

Cold nuclear fusion may be important in celestial bodies other than the Earth. Jupiter, for example, radiates about twice as much heat as it receives from the Sun. It is interesting to consider whether cold nuclear fusion in the core of Jupiter, which is probably metallic hydrogen plus iron silicate, could account for its excess heat. Heat is radiated at an approximate rate of 10^{18} watts, which could be produced by p-d fusions occurring at a rate of 10^{30} s^{-1} . Assuming a core of radius 4.6×10^9 cm, containing mostly hydrogen, with density $\sim 10 \text{ g cm}^{-3}$ and a deuteron/proton ratio of $\sim 10^{-4}$, we deduce a required p-d fusion rate of $\lambda_f \approx 10^{-19}$ fusions $\text{d}^{-1} \text{ s}^{-1}$ if all the heat derives from fusion. Catalysed nuclear fusion at this rate could be readily measured in the laboratory.

Further evidence for cold nuclear fusion in condensed matter comes from studies of ^3He and ^4He in metals. There have been several reports of high ^3He concentrations in metal crucibles and foils (H. Craig, R. Poreda, A. Nier, personal communications), consistent with *in situ* formation by cold fusion. In particular, Mamyrin *et al.*⁸ report the occurrence of patchy, high concentrations of ^3He in a number of metal foils. Electrolytic refining of the metals could have provided the appropriate conditions for the cold nuclear fusion reactions (1) and possibly (2). Among several possible explanations for the observations, the authors suggest an analogue of muon catalysis⁸.

Detection of cold-fusion neutrons

The considerations outlined above led to laboratory experiments performed at Brigham Young University to determine whether cold nuclear fusion can actually occur in condensed matter. We now report the observation of deuteron-deuteron fusion at room temperature during low-voltage electrolytic infusion of deuterons into metallic titanium or palladium electrodes. The fusion reaction (2a) is apparently catalysed by the deposition of d^+ and metal ions from the electrolyte at (and into) the negative electrode. Neutrons with an energy of ~ 2.5 MeV are clearly detected with a sensitive neutron spectrometer. The experimental layout is shown in Fig. 1.

The neutron spectrometer, developed at Brigham Young University over the past few years (ref. 9 and manuscript in preparation) has been crucial to the identification of this cold fusion process. The detector consists of a liquid organic scintillator (BC-505) contained in a glass cylinder 12.5 cm in diameter, in

which three glass scintillator plates doped with lithium-6 are embedded. Neutrons deposit energy in the liquid scintillator through multiple collisions, and the resulting light output yields energy information. As their energy decreases, the neutrons are scavenged by ^6Li nuclei, and the reaction $n + ^6\text{Li} \rightarrow t + ^4\text{He}$ results in scintillations in the glass. Pulse shapes and amplitudes from the two scintillators differ; the two distinct signals are registered by two photomultiplier tubes, whose signals are summed. A coincidence of identified signals from the two media within $20\ \mu\text{s}$ identifies an incoming neutron that has stopped in the detector.

The spectrometer was calibrated using 2.9- and 5.2-MeV neutrons generated by deuteron-deuteron interactions at 90° and 0° , respectively, with respect to a deuteron beam from a Van de Graaff accelerator. The observed energy spectra show broad structures which imply that 2.45-MeV neutrons should appear in the multichannel analyser spectrum in channels 45–150. The stability of the detector system was checked between data runs by measuring the counting rate for fission neutrons from a broad-spectrum californium-252 source.

We have performed extensive tests to verify that the neutron spectrometer does not respond preferentially in this pulse height range to other sources of radiation such as thermal neutrons. In particular, we made unsuccessful efforts to generate false 2.5-MeV neutron 'signals' by using various γ -ray and neutron sources and by turning auxiliary equipment on and off. Neutron-producing machines such as the Van de Graaff accelerators were off during all foreground and background runs.

Many background runs were made using operating cells (described below) containing standard electrodes and electrolytes, except that H_2O replaced the D_2O ; other background runs were made using both new and previously used standard cells containing D_2O plus the usual electrolyte but with no electrical current. The individual background runs were all featureless and closely followed the pattern of the integrated background shown in Fig. 2. Background rates in the neutron counter are $\sim 10^{-3}\ \text{s}^{-1}$ in the energy region where 2.5-MeV neutrons are anticipated. By comparing energy spectra from γ -ray and neutron sources we have determined that approximately one-fourth of the observed background events arise from accidental coincidences of γ -rays and three-fourths from ambient neutrons. The γ -ray background comes mainly from radioactive radium and potassium in the surrounding materials.

We attribute the ambient neutrons to cosmic-ray sources. Although the typical neutron evaporation spectrum (at birth) has a broad maximum near 2.5 MeV (ref. 10), Monte Carlo calculations show that moderation in the source medium (predominantly the shielding surrounding the detector) will wash out this structure and produce a smoothly decreasing background spectrum above 0.5 MeV, as observed.

The predicted and measured absence of structure in the spectrum of cosmic-ray-produced neutrons will not be influenced by the relatively small temporal variations that may occur in the cosmic-ray flux, such as the observed decreases that may accompany solar flares. This means that the observed peak at 2.5 MeV cannot be accounted for by ambient-neutron background variations, because, as explained below, the analysis is based on the shape of the spectra and not simply on rates. Low-energy cosmic-ray muons would be rapidly scavenged by nuclei with high atomic number, so as to reduce muon-catalysed d-d fusion to a negligible level^{2,3}. Considering volume and solid angle, the rate of production of neutrons by muons absorbed by carbon nuclei in the detector exceeds that from muons absorbed by oxygen nuclei in the electrolytic cells by a factor of ~ 60 . Thus, the presence or absence of electrolytic cells is an unimportant perturbation in the background.

During the search for suitable catalytic materials, the following (unoptimized) prescription for the electrolytic cells evolved. It began with salts typical of volcanic hot springs and included electrode-metal ions. The electrolyte is typically a mixture of $\sim 160\ \text{g}\ \text{D}_2\text{O}$ plus various metal salts in $\sim 0.1\ \text{g}$ amounts each: $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$, PdCl_2 , CaCO_3 , $\text{Li}_2\text{SO}_4 \cdot \text{H}_2\text{O}$, $\text{Na}_2\text{SO}_4 \cdot 10\text{H}_2\text{O}$, $\text{CaH}_4(\text{PO}_4)_2 \cdot \text{H}_2\text{O}$, $\text{TiOSO}_4 \cdot \text{H}_2\text{SO}_4 \cdot 8\text{H}_2\text{O}$, and a very small amount of AuCN . The pH is adjusted to ≤ 3 with HNO_3 . All 14 runs reported here began with this basic electrolyte.

Titanium and palladium, initially selected because of their large capacities for holding hydrogen and forming hydrides, were found to be effective negative electrodes. Individual electrodes consisted of $\sim 1\ \text{g}$ purified 'fused' titanium in pellet form, or $0.05\ \text{g}$ of 0.025-mm -thick palladium foils, or $5\ \text{g}$ of mossy palladium. Typically 4–8 cells were used simultaneously. The palladium pieces were sometimes re-used after cleaning and roughening the surfaces with dilute acid or abrasives. Hydrogen bubbles were observed to form on the Pd foils only after several minutes of electrolysis, suggesting the rapid absorption of

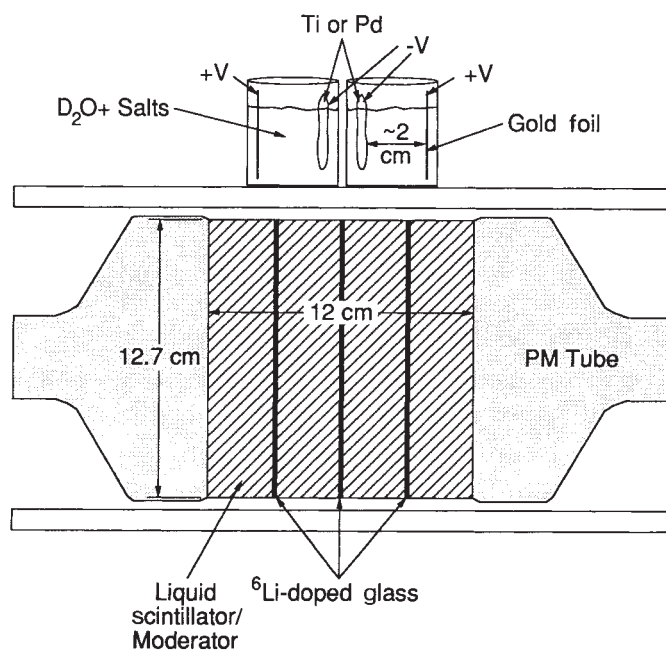


FIG. 1 Schematic diagram of the experiment. Electrolytic cells are shown on top of the neutron spectrometer.

deuterons into the foil; oxygen bubbles formed at the anode immediately. Gold foil was used for the positive electrodes. Direct-current power supplies provided 3–25 volts across each cell at currents of 10–500 mA. Correlations between fusion yield and voltage, current density, or surface characteristics of the metallic cathode have not yet been established.

Small jars, ~4 cm high and 4 cm in diameter, held ~20 ml of electrolyte solution each. The electrolytic cells were placed on or alongside the neutron counter, as shown in Fig. 1. The present cells are simple and undoubtedly far from optimum. Nevertheless, the present combination of our cells with the neutron spectrometer is sufficient to establish the phenomenon of cold nuclear fusion during electrolytic infusion of deuterium into metals.

Figure 2 shows the energy spectrum obtained under the conditions described above, juxtaposed with the (scaled) background spectrum. We acquired about twice as much background data as foreground data. Assuming conservatively that all deviations from background are statistical fluctuations, we scale the background counts by a factor of 0.46 to match the total number of foreground counts over the entire energy range shown in Fig. 2. A feature in channels 45–150 rises above background by nearly four standard deviations. This implies that our assumption is too conservative and that this structure represents a real physical effect. After re-scaling the background by a factor of 0.44 to match the foreground levels in regions just below and just above this feature, the difference plot (Fig. 3) is obtained. It shows a robust signal centred near channel 100, with a statistical significance of almost five standard deviations. A gaussian fit to this peak yields a centroid at channel 101 with a standard deviation of 28 channels, and an amplitude of 23.2 ± 4.5 counts. Both the position and width of this feature correspond to those expected for 2.5-MeV neutrons, according to the spectrometer calibration. The fact that a significant signal appears above background with the correct energy for d-d fusion neutrons (~2.5 MeV) provides strong evidence that room-temperature nuclear fusion is occurring at a low rate in the electrolytic catalysis cells.

Fusion rate determination

It is instructive to examine the fourteen individual runs which enter into the combined data discussed above. These runs were performed over the period 31 December 1988 to 6 March 1989. Figure 4 displays, for each run, the ratio of foreground count rate in the 2.5-MeV energy region to the background rate

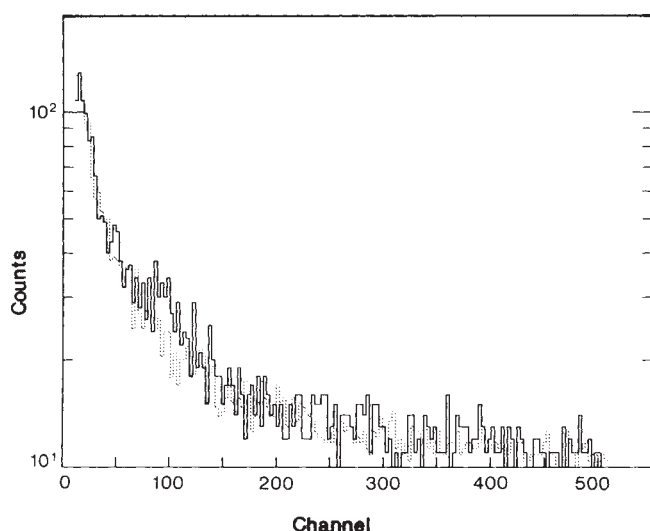


FIG. 2 Foreground (solid) and background (dashed) counts as a function of pulse height (corresponding to neutron energy) in the neutron spectrometer. Ten counts have been added to each three-channel bin for clarity of presentation.

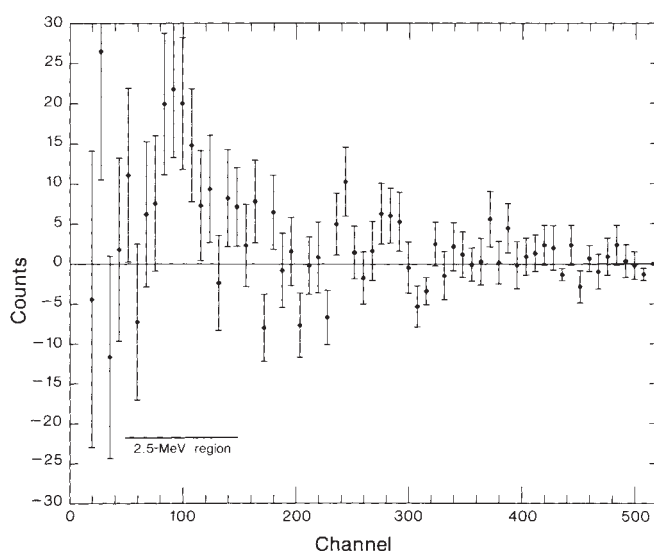


FIG. 3 Difference spectrum obtained by subtracting scaled background from the foreground. Statistical errors ($\pm 1\sigma$) are shown for each eight-channel bin.

obtained for each run. Electronic changes were made in the apparatus during the course of the experiment which altered the observed background rates, so we plot the data in terms of foreground-to-background ratios rather than absolute rates. In one set of data (runs 1 to 8) for which the system was kept as untouched as possible to avoid changes in background rates, the measured rate of detection of 2.5-MeV neutrons was $(6.2 \pm 1.3) \times 10^{-4} \text{ s}^{-1}$ above background. For this set of data, the background and foreground rates for all energies above ~3 MeV (that is, for all channels from 190 to 512) are equal, at $(1.4 \pm 0.1) \times 10^{-3} \text{ s}^{-1}$.

Run 6 is particularly noteworthy, with a statistical significance of approximately five standard deviations above background. Fused titanium pellets were used as the negative electrode, with a total mass of ~3 g. The neutron production rate increased after about one hour of electrolysis. After about eight hours, the rate dropped dramatically, as shown in the follow-on run 7. At this time, the surfaces of the titanium electrodes showed a dark grey coating. An analysis using electron microscopy with a microprobe showed that the surface coating was mostly iron, deposited with deuterons at the cathode. The same phenomenon of a decrease in the neutron signal after about eight hours of operation appears in run 13 followed by run 14. Runs 13 and 14 use the same eight electrochemical cells, and again the negative electrodes developed coatings after a few hours of electrolysis. These observations suggest the importance of surface conditions for the cold fusion process. Variations in surface conditions and electrolyte composition are anticipated during each test run because materials plate out of solution; the solution pH also changes significantly during a run. These 14 runs represent two choices of electrode material plus various operating currents. These variations may account for the fluctuations in the signal level that are evident in Fig. 4. As these runs represent a total of only ~200 signal neutrons at an average rate of ~2 per hour, it was difficult to optimize experimental conditions. This is a task for future research.

The observed 'turning off' of the signal after about eight hours may account for low signal-to-background ratios in runs 1 and 3, in that a signal that lasted for only a few hours may have been overwhelmed after a long (~20-hour) running time. When run 10 started with rates substantially above background, we stopped the run and removed half of the electrochemical cells as a test. The neutron production rate dropped off as expected (run 11). In determining the statistical significance of the data, we included runs 1, 3, 7, 11 and 14, even though we see a

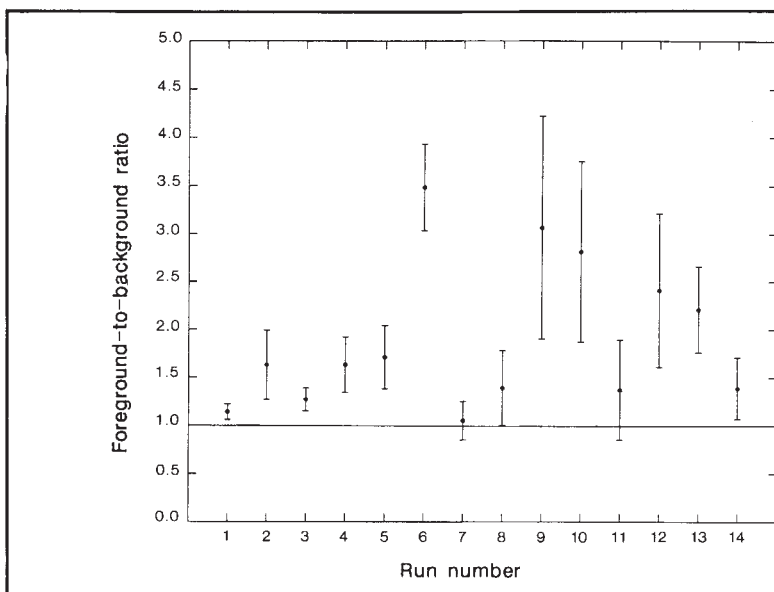


FIG. 4 Ratio of foreground rate to background rate for each run, in the 2.5-MeV energy region of the pulse-height spectrum. Statistical errors ($\pm 1\sigma$) are shown.

systematic reason for their low foreground-to-background ratios as explained above. Run 8, shown in Fig. 4, was inadvertently lost from the magnetic storage device and could not be included in Figs 2 and 3. This does not change our conclusions.

We can estimate the rate for the neutron-production branch of d-d fusion during electrolysis, specifically for run 6, as follows:

$$\text{Fusions per deuteron pair per second} = \frac{R/\varepsilon}{M \times \frac{d}{2M}} \quad (4)$$

where the observed rate of neutron detection, $R = (4.1 \pm 0.8) \times 10^{-3} \text{ s}^{-1}$, is based on foreground minus corresponding background counts in channels 45–150; the neutron detection efficiency, including geometrical acceptance, is calculated using a Monte Carlo neutron-photon transport code¹¹ to be $\varepsilon = (1.0 \pm 0.3)\%$; $M \approx 4 \times 10^{22}$ titanium atoms for 3 g of titanium; and the ratio of deuteron pairs to metal ions, $d/2M \approx 1$, is based on the assumption that nearly all tetrahedral sites in the titanium lattice are occupied, forming the $\gamma\text{-TiD}_2$ hydride. Then the estimated cold nuclear fusion rate for the neutron-production branch, by equation (4), is $\lambda_f \approx 10^{-23}$ fusions per deuteron pair per second. If most fusions take place near the surface, or if the titanium lattice is far from saturated with deuterons, or if conditions favouring fusion occur intermittently, then the inferred fusion rate must be much larger, perhaps 10^{-20} fusions per deuteron pair per second.

We note that such a fusion rate could be achieved by 'squeezing' the deuterons to about half their normal (0.74-Å) separation in molecules. That such rates are now observed in condensed matter suggests catalysed 'piezonuclear' fusion as the explanation¹. A possible cause is that quasi-electrons form in the deuterated metal lattice, with an effective mass a few times that of a free electron. Isotopes of hydrogen are known to accumulate at imperfections in metal lattices¹², and a local high concentration

of hydrogen ions might be conducive to piezonuclear fusion. Because we have not seen any evidence for fusion in equilibrated, deuterated metals or compounds such as methylamine- d_2 deuteriochloride or ammonium- d_4 chloride, we conclude that non-equilibrium conditions are essential. Electrolysis is one way to produce conditions that are far from equilibrium.

It may seem remarkable that one might influence the effective rate of fusion by varying external parameters such as pressure, temperature and electromagnetic fields, but just such effects are seen in another form of cold nuclear fusion, muon-catalysed fusion¹³.

Conclusions

The correlation of ideas regarding cold piezonuclear fusion¹ with observations of excess ^3He in metals and in geothermal areas of the Earth led to our experimental studies of fusion in electrochemical cells, which began in May 1986. Our electrolyte compositions evolved from geochemical considerations, and changed as results were observed. The presence of a fusion neutron signal was consistently reproduced, although the rate varied widely. Now that our exploratory searches have disclosed a small piezonuclear fusion effect, it remains to disentangle the factors that influence the fusion rate.

The need for off-equilibrium conditions is clearly implied by our data, and suggests that techniques other than electrochemistry may also be successful. We have begun to explore the use of ion implantation and of elevated pressures and temperatures, mimicking geological conditions. Cold nuclear fusion in condensed matter may be of interest as a novel probe of metal-hydrogen systems, including geological ones, and as a source of monoenergetic neutrons. If deuteron-deuteron fusion can be catalysed, then the d-t fusion reaction is possibly favoured because of its much larger nuclear cross-section. Although the fusion rates observed so far are small, the discovery of cold nuclear fusion in condensed matter opens the possibility, at least, of a new path to fusion energy. □

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1. Van Siclen, C. D. & Jones, S. E. *J. Phys. G.* **12**, 213–221 (1986).
2. Jones, S. E. *Nature* **321**, 127–133 (1986).
3. Rafelski, J. & Jones, S. E. *Scient. Am.* **257**, 84–89 (July 1987).
4. Craig, H., Lupton, J. E., Welhan, J. A. & Poreda, R. *Geophys. Res. Lett.* **5**, 897–900 (1978).
5. Lupton, J. E. & Craig, H. *Science* **214**, 13–18 (1981).
6. Mamyrin, B. A. & Tolstikhin, L. N. *Helium Isotopes in Nature* (Elsevier, Amsterdam, 1984).
7. Craig, H. & Lupton, J. E. in *The Sea Vol. 7* (ed. Emiliani, C.) Ch. 11 (Wiley, New York, 1981).
8. Mamyrin, B. A., Khabarin, L. V. & Yudenich, V. S. *Soviet Phys. Dokl.* **23**, 581–583 (1978).
9. Jensen, G. L., Dixon, D. R., Bruening, K. & Czirr, J. B. *Nucl. Instrum. Meth.* **220**, 406–408 (1984).
10. Hess, W. N., Patterson, H. W. & Wallace, R. *Phys. Rev.* **116**, 445–457 (1959).

11. MCNP: Monte Carlo Neutron and Photon Transport Code, CCC-200 (Version 3) (Radiation Shielding Information Center, Oak Ridge Natn. Lab., 1983).

12. Bowman, R. C. Jr in *Metal Hydrides* (ed. Bambakides, G.) 109–144 (Plenum, New York, 1981).

13. Jones, S. E. et al. *Phys. Rev. Lett.* **51**, 1757–1760 (1983).

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■ For a comment from one of the referees of this paper, please see page 711.

Differing strategies for organizing anterior and posterior body pattern in *Drosophila* embryos

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Opposing anterior and posterior morphogen systems specify the segmented body pattern of *Drosophila*. The anterior morphogen, *bicoid*, exerts a direct, instructive influence on head and thoracic pattern by triggering different outcomes according to changes in its concentration along the body. In contrast, the posterior morphogen, *nanos*, simply defines where abdominal patterning can occur by eliminating an otherwise ubiquitous repressor, *hunchback* protein, from the posterior half of the embryo. Within this *hunchback*-free domain the pattern of abdominal segments must be specified by other morphogens, possibly by shorter range gradients of the products of zygotic gap genes *Kruppel*, *knirps* and *tailless*.

THE body plan of *Drosophila* is laid down in response to opposing morphogen gradients emanating from localized sources at each pole of the embryo¹⁻³. The anterior morphogen, *bicoid* (*bcd*), appears to organize head and thoracic pattern by directly dictating the spatial expression of several subordinate signalling molecules⁴⁻⁷. In this regard, it behaves as a classical gradient morphogen, triggering a series of distinct results according to its changing concentration along the body^{8,9}. Less is known about the posterior morphogen, *nanos* (*nos*)^{3,10,11} although diffusion or transport of this molecule from the posterior pole towards the middle of the early embryo is normally essential for the development of abdominal pattern².

The *bicoid* (*bcd*)^{1,3-7} and *nanos* (*nos*)^{3,10,11} morphogens profoundly influence the patterns of expression of many subordinate signalling molecules^{4-7,12-15}. Although most of these effects are likely to be indirect, it is notable that both morphogens play early and major roles in generating restricted anterior expression of the protein product of the gap gene *hunchback* (*hb*; refs 12-14)⁵. The *hb* gene is first expressed during oogenesis and its transcripts are deposited throughout the egg¹⁷. Following fertilization, *nos* morphogen emanating from the posterior pole appears to destabilize these maternal transcripts, thereby preventing early expression of *hb* protein in the posterior half of the embryo^{5,17,18}. Shortly thereafter, *bcd* morphogen spreading from the anterior pole triggers the transcriptional activation of the *hb* gene in the anterior half of the body^{5,9,18}.

Zygotic activity of the *hb* gene under the control of the *bcd* gradient is critically required for the development of normal head and thoracic segments^{13,14}; indeed it is sufficient for normal development to occur even in the absence of the maternal *hb* contribution¹³. In contrast, the role of the *nos* morphogen in repressing posterior *hb* expression is of unknown significance. The two experiments reported here test the significance of this interaction by determining what happens when the repression of *hb* is overridden or circumvented. In the first experiment, ectopic expression of the *hb* coding sequence in the posterior half of the body is shown to cause phenotypes which mimic those caused by absence of the posterior determinant system.

Conversely, the second experiment establishes that the requirement for the posterior morphogen can be obviated by inactivating maternally derived *hb* transcripts by other means. Taken together, these experiments establish that the *nos* morphogen influences the development of abdominal pattern primarily, if not exclusively, by precluding posterior *hb* expression. Hence, *nos* appears to have a permissive rather than an instructive role in generating the posterior pattern, providing a sharp contrast to the role of *bcd* in organizing anterior pattern.

Results

Ectopic *hb* expression causes *nos* mutant phenocopies. Ectopic *hb* expression was generated by placing the coding sequence of the *hb* gene under the control of the *Drosophila hsp70* promoter¹⁹, integrating the hybrid *hsp70-hb* gene into the germ line via P-element mediated transformation²⁰, and then activating transcription of the transformed gene by exposing embryos carrying it to heat shocks at various stages of their development (see legend to Fig. 1). As shown in Fig. 1*a-c*, heat shocking *hsp70-hb* embryos during the syncytial blastoderm stage generates a pattern of posterior *hb* expression similar to that of embryos lacking the posterior determinant system. When allowed to develop to hatching, such embryos give rise to larvae lacking or having severely reduced abdominal segmentation (Fig. 2*a-c*), a phenotype characteristic of larvae derived from embryos in which the *nos* morphogen is largely or completely inactive (for example, Fig. 2*d*)^{2,3,11}. The loss of abdominal segmentation observed in both cases appears to arise in a similar fashion as indicated by the expression of the pair-rule gene *even-skipped* (*eve*)^{12,21,22}. The periodic expression of this gene is one of the earliest manifestations of segmentation. As shown in Fig. 1*d-f*, heat-shocked *hsp70-hb* embryos show an altered pattern of *eve* expression which resembles that of embryos lacking the posterior determinant system.

Thus, driving inappropriate posterior expression of the *hb* protein in wild-type embryos is sufficient to cause phenotypes which mimic those associated with absence of the posterior determinant system. This result suggests that the *nos* morphogen may normally influence abdominal segmentation solely by destabilizing maternal transcripts of the *hb* gene, thereby precluding posterior expression of *hb* protein during early embryogenesis. If so, the posterior determinant system should only be required in embryos that receive functional maternal transcripts from the *hb* gene: embryos lacking such transcripts should develop normal abdominal patterns even if the *nos* morphogen is inactive. As described below, this prediction has been tested and confirmed by using X-ray-induced mitotic recombination to remove functional *hb* transcripts from single cells during the development of the female germ line^{23,24}.

The *nos* morphogen is superfluous in embryos lacking functional maternal *hb* transcripts. Females homozygous for mutations in the *vasa* gene give rise to eggs in which the *nos* morphogen is inactive (refs 11, 25; Fig. 2*d*). These eggs also lack functional germ-cell determinants normally deposited at the posterior pole and hence give rise to agametic embryos. As shown in Fig. 3, it is possible to remove *hb* gene function from single germ cells in *vasa* females by irradiating larvae of the genotype *vas/vas*; *hb/+*. Of approximately 800 *vas/vas*; *hb/+* females irradiated

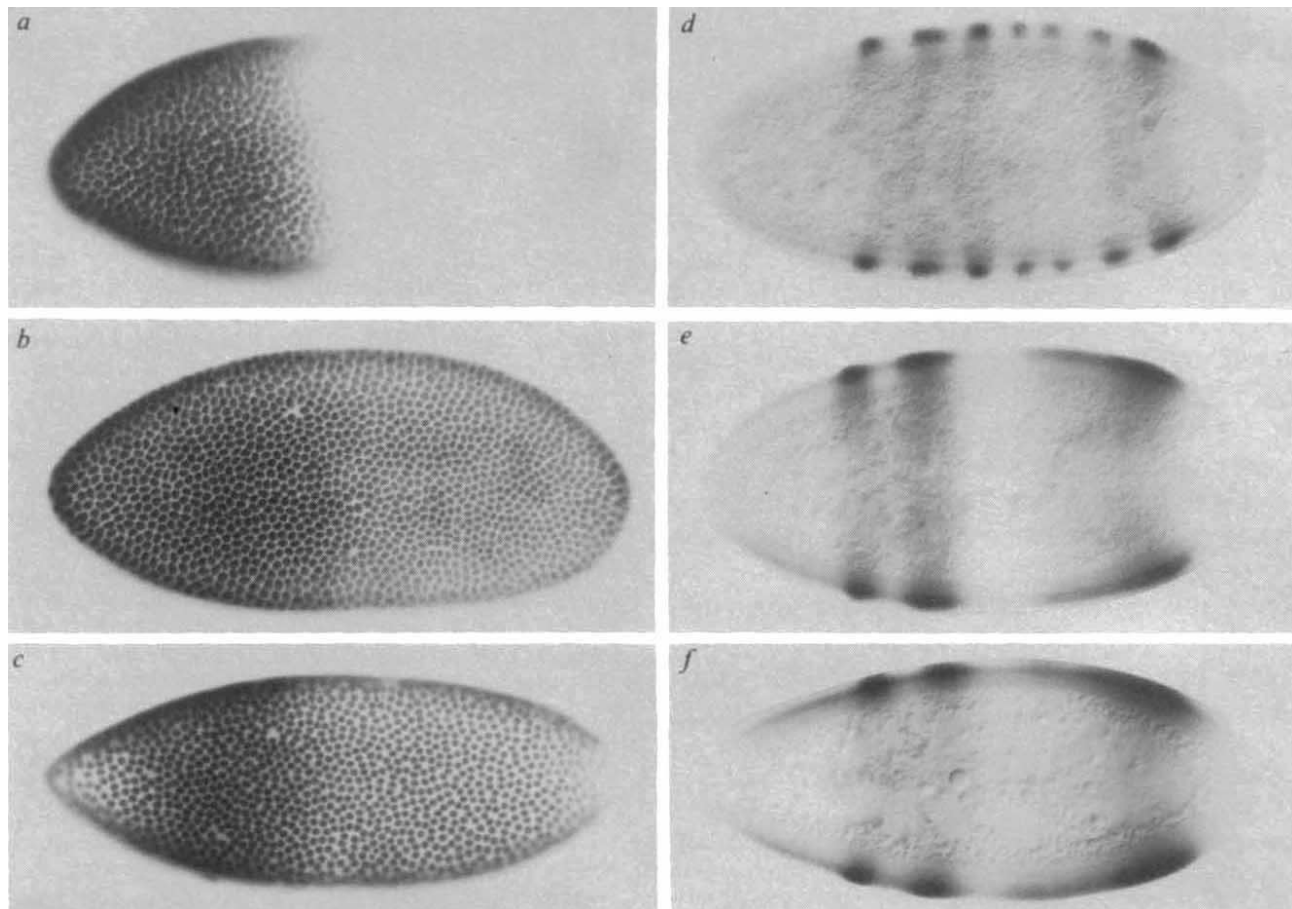


FIG. 1 Ectopic expression of the *hunchback* protein under *hsp70* control mimics the phenotype associated with absence of the posterior determinant *nanos*. *a* and *b*, Expression of *hb* protein in wild-type and *hsp70-hb* embryos 20 minutes after heat shock (15 min, 36 °C). *c*, Expression of *hb* protein in embryos derived from *vasa* females, which lack *nos* function (all embryos are in the twelfth nuclear division cycle following fertilization and are oriented with their anterior poles at the left; *vasa* mutant embryos were obtained from females homozygous for the *vasa*^{PD} mutation). Note that wild-type embryos express *hb* protein exclusively in the anterior half (under the control of the anterior morphogen, *bcd*), whereas heat-shocked *hsp70-hb* and *vasa* embryos show ectopic expression of *hb* protein in the posterior half. *d*, *e* and *f*, Expression of *eve* protein in wild-type and *hsp70-hb* embryos 60 minutes after heat shock (10 min, 36 °C), and in *vasa* embryos, respectively (all embryos are in the later portion of the fourteenth nuclear division cycle, just before completing cellularization and beginning gastrulation, and are oriented as in *a*, *b* and *c*). The protein product of the *eve* gene is normally expressed in seven stripes at this stage (numbered 1–7 in the anterior to posterior direction). In both heat-shocked *hsp70-hb* and *vasa* mutant embryos, stripes 1 and 2, which are normally associated with the gnathal and anterior thoracic segments, are present, but broader than normal; however, stripes 3–6, which are normally associated with posterior thoracic segments and the first seven abdominal segments appear to be replaced by a domain of intermediate expression which seems to be fused to an enlarged stripe 7. The extent of this broad posterior domain is variable in both heat shocked *hsp70-hb* embryos as well as in *vasa* mutant embryos, although as shown here, the overall pattern is generally similar. Note that the extent of ectopic *hb* expression under *hsp70* control is variable: the embryo in *b* shows a typical example, although *hsp70-hb* expression is often so strong that the embryos appear to express *hb* protein uniformly. Note also that heat shocks administered any time after the blastoderm stage have no detectable effects on the pattern of body segments, even though high levels of ectopic *hb* expression are induced and persist for several hours.

METHODS. The *hsp70-hb* gene was constructed as follows: a 2.4-kb *Xba*I fragment from the *hb* gene containing the entire *hb* coding sequence

preceded by 15 nucleotides at the 5' end and followed by 152 nucleotides at the 3' end was excised from a genomic clone *pE8-B1000A* (kindly supplied by D. Tautz; ref. 17), and inserted into an *hsp70* expression plasmid such that it is positioned immediately downstream of the *hsp70* promoter^{19,29} and upstream of the 3' untranslated portion of the $\alpha 1$ tubulin gene (including a putative transcriptional stop site (see ref. 30)). The resulting *hsp70-hb-tubulin* hybrid gene was then excised and inserted into the *ry*⁺ P-element vector *Carnegie 20* (ref. 31) and integrated into the genome by P-element mediated transformation²⁰. Two transformants were obtained, one of which, *HSHB-3*, contains an insert on the third chromosome. All of the experiments were performed using a stock which is homozygous for this insert. The embryos were heat-shocked as follows. Approximately staged embryos were obtained from timed egg collections, rinsed on a piece of nytex, and the nytex placed on the wet surface of an agar plate maintained at 36 °C. After 10 or 15 min (heat shocks of either duration gave indistinguishable results) the nytex was transferred to a fresh wet agar plate at 25 °C and incubated at that temperature until they were fixed for antibody staining or examined for their cuticular phenotypes (see Fig. 2). Embryos were stained for *hb* or *eve* protein as previously described³² using rat anti-*hb* and rat anti-*eve* polyclonal antisera generously provided by P. Macdonald. Virtually all *hsp70-hb* embryos stained for *hb* antigen following heat shock show moderate to high levels of uniform *hb* expression superimposed on top of the normal pattern of *hb* expression (note however that embryos heat-shocked before the syncytial blastoderm stage do not show ectopic *hb* expression; also, ectopic *hb* expression was not observed in pole cells). The expression of *hb* protein induced by heat shocking *hsp70-hb* embryos increases progressively over a 1–2 hr interval following a 10 or 15-min heat shock, and slowly dissipates over a period of several hours thereafter. Most (> 90%) of the *hsp70-hb* embryos in the late phases of nuclear cycle 14 obtained following a 10-min heat shock 60 minutes earlier show abnormal patterns of *eve* expression similar to that shown in *e*, the principal variation being the extent of the posterior domain of expression (in a minority of cases, this broad domain appeared to partially resolve into two neighbouring domains). Older sibling embryos obtained in the same collections showed normal *eve* patterns of expression and segmental morphologies.

during the first or second larval instars, at least 24 gave rise to normal larval and adult progeny when outcrossed to wild-type males. As described in the legend to Fig. 3, all of the adult progeny from these females were shown to derive from *vas/vas*; *hb/hb* germ-line clones. Thus, *vasa* mutant embryos, which lack functional *nos* morphogen, can nevertheless develop into larvae and adults having normal abdominal patterns provided that they lack functional maternal transcripts of the *hb* gene.

Discussion

The results presented here show that the primary, if not sole, role of the posterior determinant system is to prevent posterior expression of *hb* protein from maternal transcripts deposited throughout the egg. Similar conclusions have been reached independently by D. Tautz and colleagues (personal communication) on the basis of complementary experiments. These findings have several implications (see ref. 26 for a more extensive discussion of these points):

(1) Molecular role of the *nos* morphogen. The early and potentially exclusive role played by the *nos* morphogen in clearing maternal *hb* transcripts from the posterior half of the body suggests that it directly binds and destabilizes these transcripts. Both the *hsp70-hb* hybrid gene reported here, as well as an *hb-βgal* hybrid gene generated by Tautz *et al.* (personal communication) appear to be unaffected by the *nos* morphogen.

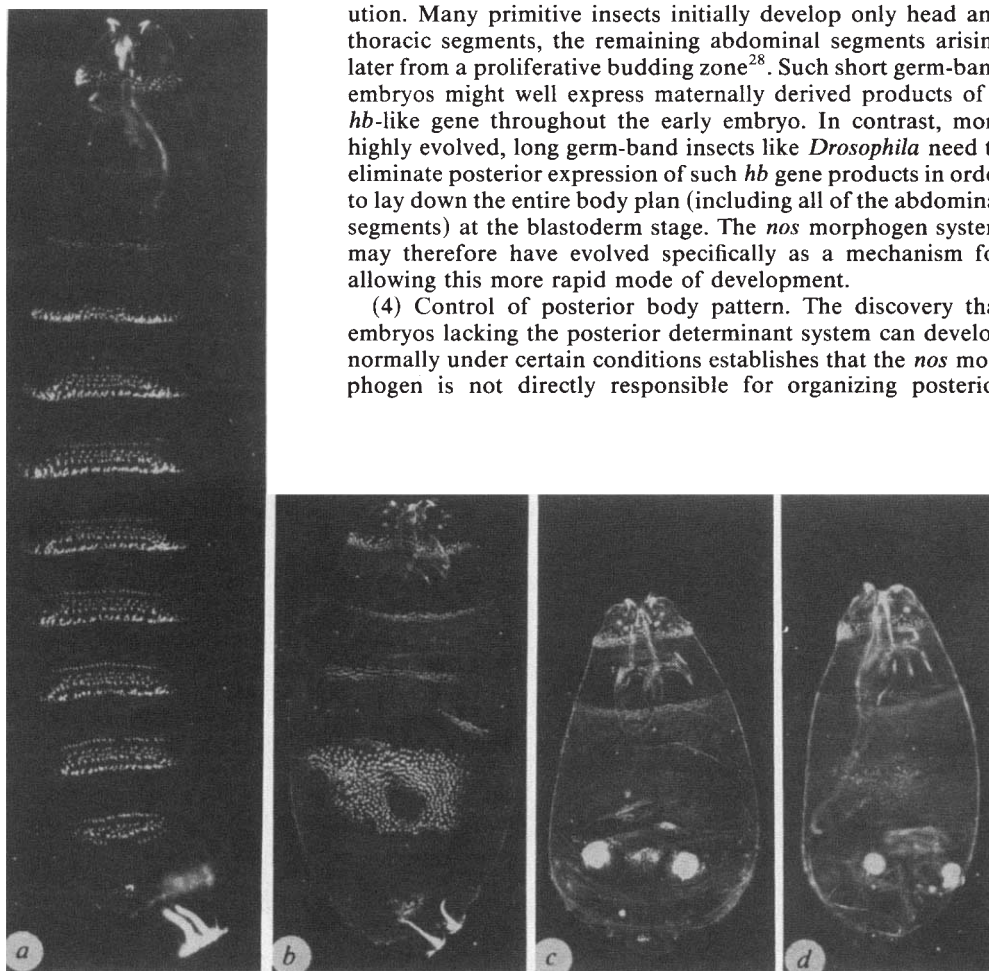
Transcripts from both of these hybrid genes lack most or all of the 3' untranslated portion of the endogenous *hb* transcript, suggesting that the target site for the *nos* morphogen may lie in this portion of the molecule.

(2) Molecular role of the *hb* protein. The posterior determinant is thought to control abdominal segmentation by controlling the regional activation of the gap gene *knirps* (*kni*)^{3,11}. This supposition was initially based on the phenotype of *kni* mutant embryos that resembles that associated with mutations which partially inactivate the *nos* morphogen, and is supported by preliminary molecular studies of U. Nauber and colleagues (unpublished data, cited in refs 15, 27). The results presented here suggest that the *nos* morphogen activates *kni* gene expression indirectly by repressing inappropriate *hb* expression in the posterior half of the body. The *hb* protein may therefore directly repress transcription of the *kni* gene. Thus, the *bcd* and *nos* morphogens may specify the subdivision of the body into distinct anterior (*hb-on*; *kni-off*) and posterior (*hb-off*; *kni-on*) patterning domains by their immediate effects on the pattern of *hb* expression, the activation of *kni* resulting by default from the absence of *hb* protein.

(3) Role of maternal *hb* expression. The present results show that maternal transcripts of the *hb* gene are not simply dispensable in normal development¹³—they are potentially deleterious. Indeed, failure to remove these unwanted transcripts from the back half of the embryo prevents abdominal differentiation. This peculiar situation may reflect an atavistic quirk in insect evolution. Many primitive insects initially develop only head and thoracic segments, the remaining abdominal segments arising later from a proliferative budding zone²⁸. Such short germ-band embryos might well express maternally derived products of a *hb*-like gene throughout the early embryo. In contrast, more highly evolved, long germ-band insects like *Drosophila* need to eliminate posterior expression of such *hb* gene products in order to lay down the entire body plan (including all of the abdominal segments) at the blastoderm stage. The *nos* morphogen system may therefore have evolved specifically as a mechanism for allowing this more rapid mode of development.

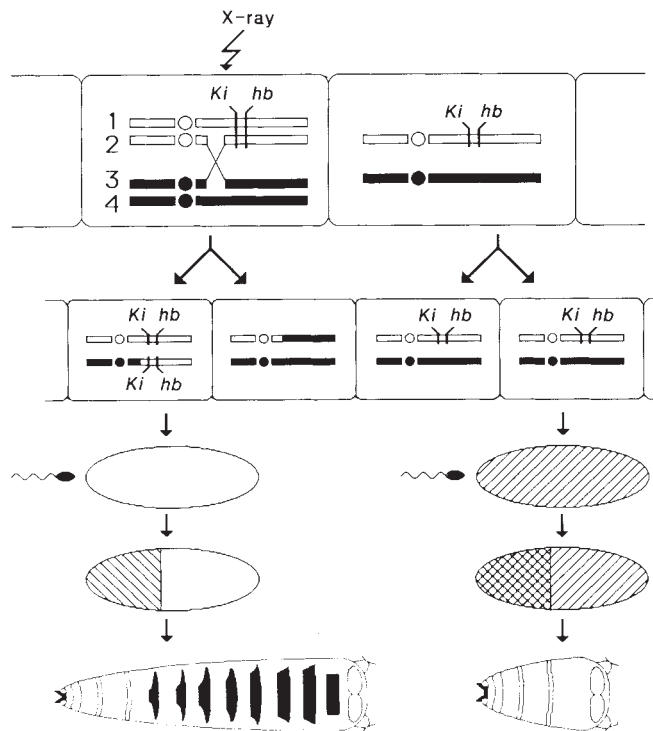
(4) Control of posterior body pattern. The discovery that embryos lacking the posterior determinant system can develop normally under certain conditions establishes that the *nos* morphogen is not directly responsible for organizing posterior

FIG. 2 Cuticular phenotypes of larvae derived from wild-type heat shocked *hsp70-hb* and *vasa* embryos. Dark-field micrographs showing the ventral aspects of larvae derived from wild-type embryos (a), heat shocked *hsp70-hb* embryos (b and c) and *vasa*^{PD} mutant embryos (d) (larvae in a and b show only the ventral aspect; larvae in c and d show superimposed ventral and dorsal aspects; all embryos are oriented with their heads at the top). Wild-type larvae differentiate eight abdominal segments which are associated with distinctive patterns of thick ventral hairs (dentical bands). These are preceded anteriorly by three thoracic segments associated with bands of fine ventral hairs and the head which includes prominent derivatives of the mouthpart segments, and followed posteriorly by terminal structures including the paired spiracles. *hsp70-hb* embryos heat-shocked approximately 105–165 min after fertilization give rise to larvae which have normal heads and anterior thoracic segments but lack most or all of the abdominal segments as shown in b and c. (Single collections of heat shocked embryos were split into two populations, one assayed for *eve* protein expression 60 minutes after heat shock, the other analysed for cuticular pattern following differentiation. Of the embryos which differentiate larval cuticle, more than 50 per cent show segmental patterns which range between the embryos shown in b and c; larvae showing wild-type cuticular patterns almost certainly developed from embryos heat shocked towards the end of the blastoderm stage or later which show normal patterns of *eve* expression.) In larvae lacking all abdominal segments, the third thoracic segment is often partially deleted and the posterior spiracles are rudimentary and fail to pair, as shown in c. These larvae closely resemble larvae derived from *vasa* mutant embryos (d) which generally lack all of the abdominal segments, show partial deletions



of the third thoracic segment and have unpaired, rudimentary spiracles. METHODS. Unhatched larvae were dechorionated with commercial bleach, transferred to a mixture of 1:1 heptane:methanol and vortexed until most were separated from the vitelline membrane. They were then rinsed in 0.1% Triton-X and mounted in a mixture of 1:1 Hoyer's mountant:lactic acid³³.

FIG. 3 Induction of homozygous *hb* clones in the germ lines of females lacking the posterior determinant system. Female first and second instar larvae of the genotype *vas^{PD}/vas^{PD}; Ki hb^{14F}/+* were irradiated with 1,000 rad to induce mitotic recombination in single germ cells as shown at the top. The *Kinked* (*Ki*) mutation is a dominant bristle marker which is positioned just proximal to the *hb* mutation on the right arm of chromosome 3 (ref. 34). Recombination between the *Ki* locus and the centromere will result in a homozygous *vas^{PD}/vas^{PD}; Ki hb^{14F}* daughter cell, provided that chromatids 1 and 3 segregate together (the *Ki* and *hb* loci are tightly linked; hence, few if any recombination events are expected to occur between them). The descendants of this cell will form a germ-line clone giving rise to eggs lacking functional maternal transcripts of the *hb* gene. The other daughter cell will be homozygous for the wild-type *Ki* and *hb* alleles and, like the surrounding *vas^{PD}/vas^{PD}; Ki hb^{14F}/+* cells, will give rise to eggs with ubiquitous, functional *hb* transcripts. Fertilization of *vas* eggs containing functional *hb* transcripts should generate phenotypically mutant embryos because the *vas* mutation prevents activity of the posterior determinant *nos*, thereby allowing the maternal *hb* transcripts (hatching) to persist and give rise to inappropriate posterior expression of the *hb* protein, as shown. Conversely, removing the wild-type allele of *hb* from the germ line via mitotic recombination should abolish this inappropriate posterior expression, whereas zygotic activation of the paternal *hb* gene (opposite hatching) under the control of the *bcd* gradient should suffice to generate the necessary anterior domain of *hb* expression. Note that the *vas^{PD}* mutation also prevents the formation of posterior polar granules associated with localized germ-cell determinants; consequently all of the progeny from *vas^{PD}/vas^{PD}; Ki hb^{14F}/+* females are agametic, irrespective of the presence or absence of functional maternal *hb* transcripts. Note also that virtually all larvae derived from these females show a less extreme mutant phenotype than that observed in larvae derived from *vas^{PD}/vas^{PD}* mothers which carry two functional copies of the *hb* gene. In particular, they generally form a single band of abdominal denticles between the thorax and posterior terminalia in contrast to standard *vas^{PD}* mutant embryos (for example, Fig. 2d) which do so only occasionally. This slight degree of phenotypic rescue probably results directly from the lower maternal *hb* gene dosage in *vas^{PD}/vas^{PD}; Ki hb^{14F}/+* females, as completely eliminating the maternal *hb* contribution, as described below, fully rescues abdominal segmentation. Approximately 800 female flies obtained from irradiated *vas^{PD}/vas^{PD}; Ki hb^{14F}/+* larvae were outcrossed in small groups of 10–20 flies to wild-type males. Of the 70 such group matings which were performed, 24 gave rise to small numbers of wild-type larvae which developed in adult flies with normal abdominal patterns; all the rest produced only *vas* mutant larvae (nine of these 24 matings gave rise to single surviving flies, six gave rise to 2–3 survivors, and eight gave rise to four or more survivors). A total of 71 such adult flies were obtained: all were phenotypically *Ki* as was to be expected if they arose from mitotic recombination. In addition, all of the flies which survived



long enough to be tested (55/71) were sterile, and in fact agametic, as was to be expected if they arose from *vas* mutant eggs. Finally, in two cases it was possible to identify the single female fly generating phenotypically wild-type larvae; in both cases, all but the few surviving progeny developed as *vas* mutant larvae. The phenotypic properties of the surviving flies therefore establish that these progeny arose from homozygous *Ki hb^{14F}/Ki hb^{14F}* clones in the germ lines of their homozygous *vas^{PD}/vas^{PD}* mothers. METHODS. Surviving flies were tested for fertility by outcrossing them singly to flies of the opposite sex for 5–7 days. Their ovaries or testes were then removed and examined under phase and Nomarski optics for the presence of germ cells. The frequency of clones obtained (~1 clone per 25 flies) is similar to that obtained in other studies (see for example, ref. 24); given that 24/70 group matings produced surviving adults, it is likely that more than one female contained a clone in some of these matings.

pattern. Instead, activity of the *nos* morphogen appears only to define the region in which abdominal segmentation will occur. Other spatial determinants must therefore be responsible for organizing pattern within this domain. One possibility is that the overlapping distributions of the products of the gap genes *kruppel*, *kni* and *tailless*^{6,12,16,27} provide a series of short-range

gradients which govern the subdivision of the posterior half of the embryo into particular abdominal segments. This strategy of controlling body pattern would differ significantly from that operating in the anterior half of the embryo where the graded distribution of a single molecular species, *bcd*, provides a coherent system of cues organizing the global pattern. □

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1. Frohnhofer, H. G. & Nüsslein-Volhard, C. *Nature* **324**, 120–125 (1986).
2. Lehmann, R. & Nüsslein-Volhard, C. *Cell* **47**, 141–152 (1986).
3. Nüsslein-Volhard, C., Frohnhofer, H. G. & Lehmann, R. *Science* **238**, 1675–1681 (1987).
4. Frohnhofer, H. G. & Nüsslein-Volhard, C. *Genes Dev.* **1**, 880–890 (1987).
5. Tautz, D. *Nature* **332**, 281–284 (1988).
6. Gaul, U. & Jäckle, H. *Cell* **51**, 549–555 (1987).
7. Mlodzik, M. & Gehring, W. *Development* **101**, 421–435 (1987).
8. Driever, W. & Nüsslein-Volhard, C. *Cell* **54**, 95–104 (1988).
9. Struhl, G., Struhl, K. & Macdonald, P. M. *Cell*, submitted.
10. Sander, K. & Lehmann, R. *Nature* **335**, 68–70 (1988).
11. Lehmann, R. *Development* **104** Suppl., 17–27 (1988).
12. Nüsslein-Volhard, C. & Wieschaus, C. *Nature* **287**, 795–801 (1980).
13. Lehmann, R. & Nüsslein-Volhard, C. *Dev. Biol.* **119**, 402–417 (1987).
14. Bender, M., Turner, F. R. & Kaufman, T. C. *Dev. Biol.* **119**, 418–432 (1987).
15. Nauber, U. et al. *Nature* **336**, 489–492 (1988).
16. Strecker, T. R., Kongsuwan, K., Lengyel, J. A. & Merriam, J. R. *Dev. Biol.* **113**, 64–71 (1986).
17. Tautz, D. et al. *Nature* **327**, 383–389 (1987).
18. Schröder, C., Tautz, D., Seifert, E. & Jäckle, H. *EMBO J.* **7**, 2881–2887 (1988).
19. Lis, J. T., Simon, J. A. & Sutton, C. A. *Cell* **35**, 403–410 (1983).
20. Rubin, G. M. & Spradling, A. C. *Science* **218**, 348–353 (1982).
21. Macdonald, P. M., Ingham, P. & Struhl, G. *Cell* **47**, 721–734 (1986).

22. Frasch, M., Hoey, T., Rushlow, C., Doyle, H. & Levine, M. *EMBO J.* **6**, 749–759 (1987).
23. Wieschaus, E. & Szabad, J. *Dev. Biol.* **68**, 29–46 (1979).
24. Erdelyi, M. & Szabad, J. *Genetics* (in the press).
25. Schüpbach, T., Wieschaus, E. *Wilhelm Roux's Arch. Dev. Biol.* **195**, 302–317.
26. Struhl, G. *Cellular basis of morphogenesis* (Ciba Foundation Symp. 144) 65–91 (Wiley, Chichester, 1989).
27. Ingham, P. *Nature* **335**, 25–34 (1988).
28. Anderson, D. T. *Developmental Systems: Insects* Vol. 1 (Academic, London, 1972).
29. Struhl, G. *Nature* **318**, 677–680 (1985).
30. Lawrence, P. A., Johnston, P., Macdonald, P. M. & Struhl, G. *Nature* **328**, 440–442 (1987).
31. Rubin, G. M. & Spradling, A. C. *Nucleic Acids Res.* **11**, 6341–6351 (1983).
32. Macdonald, P. M. & Struhl, G. *Nature* **324**, 537–545 (1986).
33. Struhl, G. *Nature* **308**, 454–457 (1984).
34. Lindely, D. L. & Grell, E. L. *Carnegie Inst. Wash. Publ.* **627** (1968).

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Detectability of gravitational microlensing in the quasar QSO2237+0305

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THE quasar QSO2237+0305 is unique in being located almost exactly behind the centre of a bright nearby galaxy, of magnitude 14.5 and redshift 0.039 (ref. 1). Recent observations²⁻⁴ show that the quasar is split into four images with very similar spectra, strongly suggesting gravitational lensing by the foreground galaxy. We show here that compact objects within the galaxy, such as stars, lying near the line of sight, can cause transient microlensing effects. Rapid changes owing to transverse motions of quasar, galaxy and observer turn out to be more likely in QSO2237+0305 than in any other system. The time delays between the four macro-images are about a day or less³, so intrinsic variations should show up 'simultaneously' in all images, and a change in observed luminosity ratios would be direct proof of microlensing. QSO2237+0305 is the only known system where such unequivocal proof is possible, making it a key object in assessing the general importance of microlensing.

Theoretical models^{3,5} that assume a smooth distribution of matter in the foreground galaxy fit the observations fairly well, and we have developed a method whereby microlensing effects from compact objects near the line of sight can be estimated from the macrolensing model. For reasonable choices of system parameters and a quasar radius $R_q < 0.05 (M/M_\odot)^{1/2}$ pc, where M is the mass of the object causing microlensing, we expect changes larger than $0.05 (M_\odot/M)^{1/2}$ mag per year in at least one of the images. For $R_q < 0.005 (M/M_\odot)^{1/2}$ pc, high-amplification events should occur about once every three years in one of the images, and would lead to a microlensing amplification of more than a factor of two on a timescale of one year or less.

Microlensing by stars close to the light path has been discussed intensively over the past decade⁶⁻¹³. It is possible that gravitational lensing, and particularly microlensing, is affecting a large fraction of the distant quasars. If so, our concepts of quasar structure, quasar evolution, quasar statistics, and mass distribution in the Universe (abundance of compact masses between $10^{-3} M_\odot$ and $10^3 M_\odot$) must clearly be revised. From the observational point of view there are some indications of variability arising from microlensing (ref. 14, C. Vanderriest *et al.*, preprint, and R. E. Schild, personal communication), but there is no firm evidence at present. To assess the general importance of microlensing, the most promising systems for such effects should be investigated first. These systems are the gravitationally lensed quasars with several macro-images, for which enough mass is 'between' the images¹⁵ that a large optical depth for microlensing (τ) usually results. Typically one finds a τ larger than 0.5ε in some of the images, where ε is the fraction of the deflector mass that is in stars. Furthermore, the discrimination between intrinsic and microlensing variability is much simpler in such systems.

Among the lensed quasars with several macro-images the quadruple QSO2237+0305 (refs 2, 3) is the most promising system, because the deflector is about ten times closer than in other known systems. Therefore the Einstein radius ζ_0 of a single star with mass M in the deflecting galaxy, projected into the source plane, is (with redshift of the deflector $z_d = 0.039$, redshift of the quasar $z_q = 1.7$, Hubble constant $H_0 = 75 \text{ km s}^{-1} \text{ Mpc}^{-1}$ and cosmological deceleration parameter $q_0 = 0$)

$$\zeta_0 \approx 0.05 \sqrt{M/M_\odot} \text{ pc} \quad (1)$$

which is about three times larger than for other known lens systems. As smearing effects strongly reduce the effect of microlensing when the quasar radius R_q is larger than ζ_0 , such effects are much less probable for QSO2237+0305. Also favourable is the fact that we expect a much larger transverse velocity V between the (anti-)caustics and the source because of the large distance ratio ($D_q/D_d \approx 11$). We find that

$$V = \frac{v_q}{(1+z_q)} - \frac{D_q}{D_d} \frac{v_d}{(1+z_d)} + \frac{D_{dq}}{D_d} \frac{v_{\text{obs}}}{(1+z_d)} \quad (2)$$

where D_d , D_q and D_{dq} are apparent size distances and v_d , v_q and v_{obs} are the transverse velocities involved. With v_d and $v_{\text{obs}} \approx 400 \text{ km s}^{-1}$ we expect $V \approx 6,000 \text{ km s}^{-1}$, which is about a factor of 10 larger than for other known systems. This results in a reduced characteristic timescale for microlensing of

$$\Delta t_0 = \zeta_0/V \approx 8 \sqrt{M/M_\odot} \frac{6,000 \text{ km s}^{-1}}{V} \text{ yr} \quad (3)$$

and more rapid changes in the observed luminosity of individual images. For $R_q \leq 0.1 \zeta_0$, that is, $R_q < 0.005 \sqrt{M/M_\odot} \text{ pc}$, a pronounced asymmetric peak in the light curve occurs when the source crosses a (micro-) caustic. For such high-amplification events (HAEs) we expect a maximum amplification owing to microlensing of

$$A_{\text{max}} \approx \sqrt{\zeta_0/R_q} \approx 2.5 (M/M_\odot)^{1/4} (0.005 \text{ pc}/R_q)^{1/2} \quad (4)$$

on a timescale of

$$\Delta t_h = R_q/V \approx (R_q/0.005 \text{ pc}) (6,000 \text{ km s}^{-1}/V) \text{ yr} \quad (5)$$

With macro-lens modelling (homogeneous deflector) it is possible to determine the projected surface mass density σ_i and the shear parameter γ' at all image positions. To estimate microlensing effects, however, we need to know the normalized surface density σ in stars^{10,11} or equivalently the optical depth τ for microlensing¹⁶,

$$\sigma = \frac{\sigma_s}{1 - \sigma_c} = \tau \quad (6)$$

and the normalized shear parameter¹¹

$$\gamma = \frac{\gamma'}{1 - \sigma_c} \quad (7)$$

Here σ_s is the projected smoothed-out surface mass density in stars, and σ_c is the projected continuous surface mass density, both in units of the critical surface mass density σ_0 :

$$\sigma_0 = \frac{c^2}{4\pi G} \frac{D_q}{D_d D_{dq}} \quad (8)$$

With these parameters we can write the lens equation, which defines the mapping from the deflector plane onto the observer (or source) plane, in the normalized form (see ref. 11 for a more detailed discussion)

$$\zeta = \text{sign}(\sigma) \mathbf{S}(\mathbf{z}) + \begin{pmatrix} 1 + \gamma & 0 \\ 0 & 1 - \gamma \end{pmatrix} \mathbf{z} \quad (9)$$

where the first term represents the effect of stars close to the light path and the second term represents the large-scale field of the deflector as a whole. Note that the length unit in the source plane is now the projected Einstein radius (compare equations (1)). If the stars had been smeared out, the amplification of one macro-image would have been

$$A_G = |(1 - \sigma_c)^2 (1 - \sigma + \gamma)(1 - \sigma - \gamma)|^{-1} \quad (10)$$

Assuming a fraction ε of the mass to be contained in stars ($\sigma_s = \varepsilon \sigma_i = \varepsilon(\sigma_s + \sigma_c)$) we can calculate σ and γ from equations (6) and (7) if σ_i and γ' are known. By eliminating ε , we find a simple relation between σ and γ :

TABLE 1 Assumed values for σ_i and γ' for the four images

	A	B	C	D
σ_i	0.36	0.45	0.88	0.61
γ'	0.44	0.28	0.55	0.66

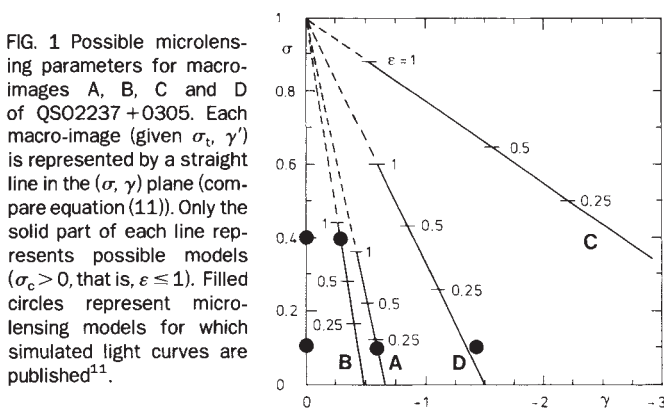
$$\sigma = 1 + \frac{\sigma_i - 1}{\gamma'} \gamma \quad (11)$$

For a given macro-image (given σ_i , γ'), this represents a straight line in the (σ, γ) diagram.

Theoretical macrolensing models of QSO2237+0305 which agree well with the observations have been presented recently by several groups^{3,5}. The quantities Amp_{cal} and τ_{lens} used in ref. 3 correspond to A_G and σ_i used in this paper (compare equation (10) and ref. 11). It is now a simple task to determine γ' (and σ_i) for all four images, and the values are given in Table 1. We have here made use of the fact that images C and D have negative parities ($\sigma_i + |\gamma'| > 1$) and that images A and B therefore must have positive parities. Note that the above discussion uses differently orientated coordinate systems for all four images.

The determination of σ_i and γ' is not possible from the data given in ref. 5 because τ_{lens} is not given; because of the similarity of the solutions, however, we expect similar parameter values. Our test calculations also indicate that the parameter values based on ref. 3 are reasonably accurate, and we therefore adopt the values given in Table 1.

In Fig. 1 the corresponding $\sigma(\gamma)$ relations (compare equation (11)) are shown for all images, with ε values indicated for some



selected points. All the straight lines go through the point $\sigma = 1$, $\gamma = 0$; note, however, that only the solid parts of the lines correspond to possible models ($\sigma_c > 0$, that is, $\varepsilon \leq 1$). Also shown in Fig. 1 are models, with equal masses in all stars, for which light curves have been presented in ref. 11. Three of these models (σ, γ) = (0.1, -0.6), (0.4, -0.4) and (0.1, -1.4), which were denoted A3, A4 and B1 respectively in ref. 11—are of particular interest here as they are very close to possible microlensing models of one of the images. The two models with $\sigma = 0.1$ should yield a reliable lower limit to microlensing variability of images A, B and D, because their ε values (0.17 and 0.08) are smaller than expected in reality, and the variability increases with increasing ε for a given σ_i and γ' . This lower limit should also apply for image C, as it has a much larger σ than the other images for the same ε .

From the 'normalized' light curve of the two $\sigma = 0.1$ models presented in ref. 11, we find, for about 50% of the time, a rate of change larger than 0.2 mag per normalized unit ζ_0 for all source sizes calculated, that is, for R_q equal to 0.011 ζ_0 , 0.1 ζ_0 and 0.9 ζ_0 respectively. For about 30% of the time the rate of

change is even larger than 0.4 mag per normalized unit. For $R_q > \zeta_0$ it is clear, however, that such rapid variations occur much less frequently with increasing R_q .

To obtain time-dependent light curves we need to know the time Δt_0 taken to move transversely by one normalized length unit. We use the estimate given in equation (3) (note that a relatively small σ_c (~ 0.3) has been assumed; compare Table 3 in ref. 11), and we find that two macro-images are expected to vary at a rate greater than $0.025 \sqrt{M_\odot/M}$ ($V/6,000 \text{ km s}^{-1}$) magnitudes per year, and one of them at a rate greater than $0.05 \sqrt{M_\odot/M}$ ($V/6,000 \text{ km s}^{-1}$) magnitudes per year if R_q is smaller than $0.05 \sqrt{M/M_\odot}$ pc. Such independent variations in the four images should not be difficult to demonstrate, because they would lead to variations of the observed luminosity ratios. This would be a proof of microlensing because intrinsic variations would show up 'simultaneously' in the four images as a result of the small time delay (about one day or even less³), and could not lead to variations in the observed luminosity ratios. Note that QSO2237+0305 is the only known system that offers the possibility of such an unequivocal proof.

From the simulated light curves in ref. 11 we estimate the frequency of high-amplification events to be $N_{\text{HAE}} \approx 0.3 \sqrt{M_\odot/M}$ ($V/6,000 \text{ km s}^{-1}$) per year in one of the images. Accurate observations of the luminosity during an HAE can give valuable information on the structure and size of the source¹³. QSO2237+0305 is the most promising object for such an investigation, because 1) smearing effects are less probable than in other systems, 2) N_{HAE} is larger and 3) intrinsic variability and microlensing can easily be separated in this system. Regular monitoring of QSO2237+0305 (on the order of once a week) should therefore be given high priority. $\square$

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- Huchra, J. et al. *Ast. J.* **90**, 691–696 (1985).
- Yee, H. K. C. *Ast. J.* **95**, 1331–1339 (1988).
- Schneider, D. P. et al. *Astr. J.* **95**, 1619–1628 (1988).
- DeRobertis, M. M. & Yee, H. K. C. *Astrophys. J.* **332**, L49–L53 (1988).
- Kent, S. M. & Falco, E. E. *Astr. J.* **96**, 1570–1574 (1988).
- Chang, K. & Refsdal, S. *Nature* **282**, 561–567 (1979).
- Gott, J. R. *Astrophys. J.* **243**, 140–146 (1981).
- Canizares, C. R. *Astrophys. J.* **263**, 508–517 (1982).
- Chang, K. & Refsdal, S. *Astr. Astrophys.* **132**, 168–178 (1984).
- Paczynski, B. *Astrophys. J.* **301**, 503–516 (1986).
- Kayser, R., Refsdal, S. & Stabell, R. *Astr. Astrophys.* **166**, 36–52 (1986).
- Schneider, P. & Weiss, A. *Astr. Astrophys.* **171**, 49–65 (1987).
- Grieger, B., Kayser, R. & Refsdal, S. *Astr. Astrophys.* **194**, 54–64 (1988).
- Foy, R., Bonneau, D. & Blazit, A. *Astr. Astrophys.* **149**, L13–L16 (1985).
- Borgeest, U. *Astrophys. J.* **309**, 467–471 (1986).
- Ostriker, J. P. & Vietri, M. *Nature* **318**, 446–448 (1985).

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Stratospheric nitric acid vapour measurements in the cold Arctic vortex: implications for nitric acid condensation

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STRATOSPHERIC nitric acid condensation has recently been proposed^{1,2} to take place in the cold winter polar vortex, priming it for chlorine-catalysed ozone destruction. This idea has been developed subsequently^{3–8}. Condensation, thought to proceed via a heterogeneous heteromolecular mechanism involving stratospheric water vapour and pre-existing $\text{H}_2\text{SO}_4/\text{H}_2\text{O}$ aerosols, which serve as condensation nuclei, leads to solid nitric acid trihydrate

(NAT; $\text{HNO}_3 \cdot 3\text{H}_2\text{O}$) aerosols. It is expected^{1,2,8} that the condensation temperature, $T_c(\text{NAT})$, is significantly greater than that of water-ice, $T_c(\text{H}_2\text{O-ice})$, given the stratospheric abundances of gaseous nitric acid and water and the thermodynamic properties of stratospheric aerosols⁸. Knowledge of $T_c(\text{NAT})$ is important because it determines the spatial and temporal extent of NAT aerosols and hence possibly of polar ozone destruction. Here we report *in situ* measurements of the detailed height distribution of gaseous nitric acid in the cold arctic vortex, using a balloon-borne technique⁹⁻¹⁶, which offers a much better altitude resolution (30 m) than previous satellite measurements^{17,18}. Our data set an upper limit to $T_c(\text{NAT})$ of 195 K at 23 km, and are consistent with model predictions based on the present gaseous nitric acid data, typical water vapour abundances and thermodynamic data⁸ of macroscopic NAT mixtures. However, our upper limit to $T_c(\text{NAT})$ is markedly lower than some of the early model estimates^{1,2}.

The ACIMS instrument used for these measurements has been described previously⁹⁻¹⁶. The present limit for gaseous nitric acid detection is about 1×10^7 HNO_3 molecules per cm^3 , far lower than typical stratospheric nitric acid concentrations at heights below 30 km ($>1 \times 10^9 \text{ cm}^{-3}$). The accuracy of the gaseous nitric acid concentrations measured here is $\pm 50\%$ and the precision is $\pm 20\%$. An important aspect of the experiment is the possibility of perturbation of the nitric acid measurements by NAT aerosols, which may evaporate inside the flow tube of our instrument. Careful simulation and atmospheric test measurements¹⁶, including also aircraft flights in the lower stratosphere, have shown that significant evaporation of stratospheric $\text{H}_2\text{SO}_4/\text{H}_2\text{O}$ aerosols can be induced only by massive additional heating of the gas stream. In our present measurements, however, the gas temperature inside the flow tube exceeds the ambient gas temperature by not more than a few degrees, therefore ruling out extensive NAT aerosol evaporation. The measurements reported here were part of the CHEOPS 2 expedition, which took place in January–February 1988 in the Arctic, mainly at ESRANGE, about 50 km east of Kiruna in Northern Sweden. During this expedition we carried out three balloon flights. One of these (B-88-2; 1 February 1988) was performed under conditions of particularly low stratospheric temperature and the results of just this flight are reported here. The balloon reached a maximum altitude of 31 km and ACIMS measurements were made on ascent between 13 and 31 km and on descent between 31 km and 5 km.

Figure 1 shows the time evolution of stratospheric temperatures in the core of the polar vortex and over ESRANGE at the 30 hPa pressure level. The height distribution of the temperature, measured by a sensor mounted 5 m above the balloon gondola, is depicted in Fig. 2. The accuracy of the temperature measurement is about ± 0.3 K. The coldest layers (193 K) were found around 23 km and 26 km. The measured height distribution of nitric acid vapour is given in Fig. 3a along with previous satellite data. Our data, with a resolution of 30 m, are generally consistent with satellite infrared measurements, which offer an altitude resolution of only 3,000–5,000 m. Our data are somewhat higher than previous model predictions^{19,20} based only on gas-phase chemistry (also shown in Fig. 3a). Our total vertical column nitric acid density of $1.5 \times 10^{16} \text{ cm}^{-2}$ is consistent with previous ground-based column measurements performed with infrared techniques²¹⁻²³ in the winter polar vortex. Figure 3b shows a portion of the nitric acid profile (around 23 km) with the maximum possible altitude resolution. Interestingly, no pronounced local minimum is seen at the heights where the lowest temperatures occurred. Some fine structure is observed, which may also be due to transport effects. We conclude from our data that $T_c(\text{NAT})$ must have been equal to or lower than 195 K. If the temperature falls below $T_c(\text{NAT})$ by only 2 K one may expect⁸ gaseous nitric acid to become depleted by about a factor of four. We cannot exclude the possibility that some nitric acid condensation had already taken place, but we can be sure that $T_c(\text{NAT})$ is no more than 2 K greater than the measured T .

Three days before our balloon flight, a search for stratospheric layers with enhanced aerosol backscattering was carried out by Poole *et al.*²⁴ using an aircraft-borne lidar (laser radar) (Fig. 1). On this flight, enhanced lidar-backscattering was observed only close to the centre of the vortex between Spitsbergen and Greenland, where the lowest temperature around 23 km was ~ 190 K. The observed excess aerosol layer was located around 23 km and was very thin (300 m).

Using the nitric acid measurements, we may estimate the height variation of $T_c(\text{NAT})$, on the basis of laboratory measurements⁸ of macroscopic NAT mixtures and taking for the stratospheric abundance of water vapour a typical value of 5 p.p.m.v. (as measured by satellite-borne remote-sensing techniques²⁵ in the winter Arctic vortex around 20 km altitude). The resulting

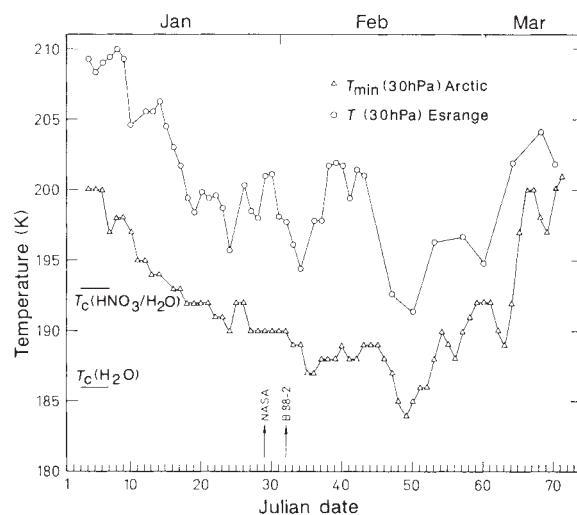


FIG. 1 Time evolution of stratospheric temperatures in the Arctic during winter and early spring of 1988. Condensation temperatures at 30 hPa for pure $\text{H}_2\text{O-ice}$ and NAT are indicated. Also marked are dates of our balloon flight B88-2 and the NASA aircraft flight. See text for details. The Arctic temperatures were provided by the Meteorological Institute of the Free University of Berlin (K. Labitzke *et al.*, personal communication).

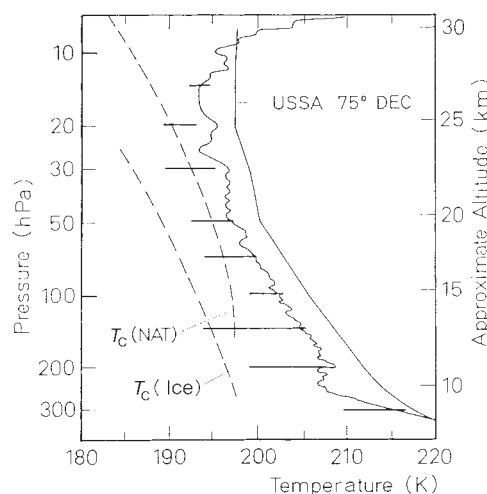


FIG. 2 Temperature profile measured on our balloon flight B88-2. For comparison we also show the US Standard Atmosphere zonally averaged temperatures for 75°N in December during cold conditions, and ranges for minimum temperatures measured in January and February over northern Sweden in 1982, 1983 and 1984 (horizontal bars). Also shown are model profiles for condensation temperatures for the NAT and $\text{H}_2\text{O-ice}$ systems, calculated using our measured nitric acid abundance and a typical water vapour abundance of 5 p.p.m.v. (dashed curves).

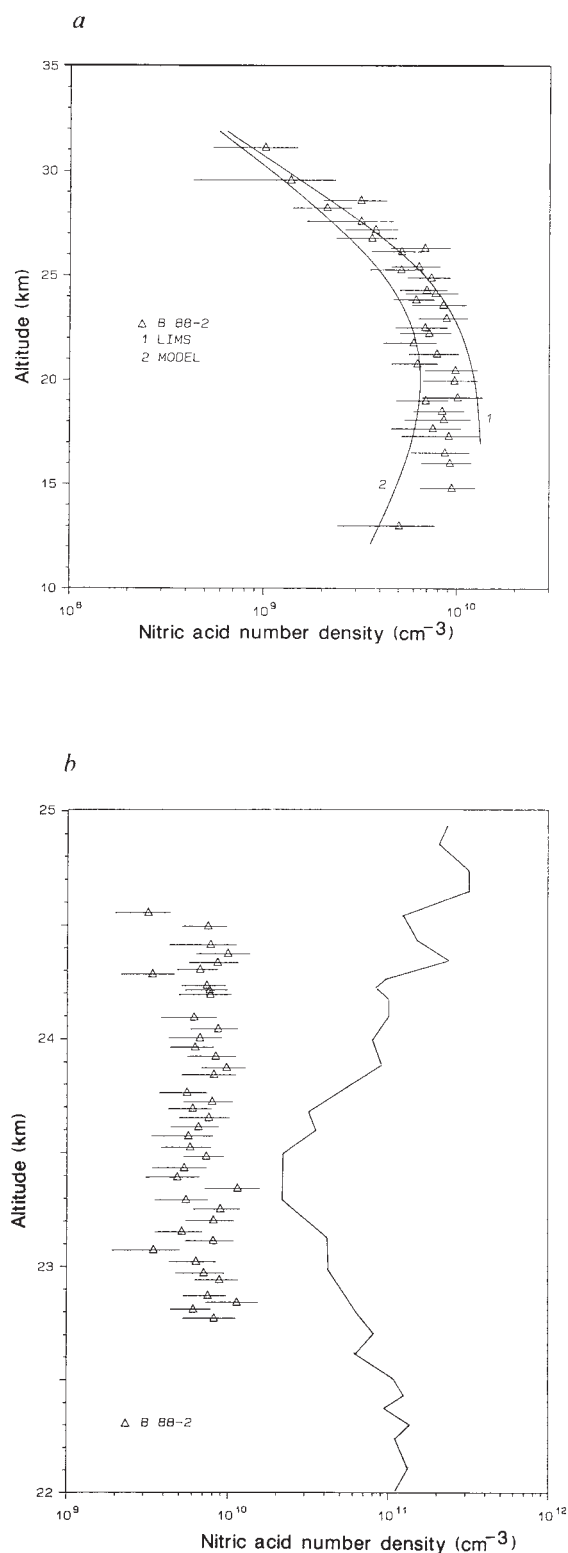


FIG. 3 *a*, Altitude distribution of concentrations of gaseous nitric acid measured by ACIMS on flight B88-2 (1 February 1988) in the cold Arctic winter vortex (the altitude is a geometric altitude measured by radar). For comparison, zonally averaged LIMS data^{9,10} for December at 70°N are also given (curve 1). Comparison is also made with theoretical model calculations^{19,20} for December at 70°N (in which heterogeneous formation of HNO₃ is neglected) (curve 2). *b*, Portion of the measured gaseous HNO₃ concentration profile shown in *a* but reprocessed to give the maximum possible height resolution. For comparison, model equilibrium saturation concentrations for HNO₃ over NAT are also shown, assuming a typical H₂O vapour mixing ratio of 5 p.p.m.v. and using the measured atmospheric temperature profile (B88-2).

height distribution of $T_c(\text{NAT})$ is shown in Fig. 2, along with $T_c(\text{H}_2\text{O-ice})$. We note, however, that water vapour abundance is a critical factor. Assuming a water vapour abundance of only 2.5 p.p.m.v., $T_c(\text{NAT})$ becomes lower by about 3 K; with a value of 7.5 p.p.m.v., $T_c(\text{NAT})$ increases by ~ 1 K (ref. 8). Hence, local and temporal variations of water vapour would probably induce large local and temporal variations in $T_c(\text{NAT})$ (of up to 4 K). Therefore, the T_c values for both NAT and pure water-ice (shown in Fig. 2) probably represent only approximate figures. The general decrease of $T_c(\text{NAT})$ with increasing altitude is due to the decrease in concentrations of both HNO₃ and H₂O. The steeper decrease above ~ 25 km reflects the steep decrease of the HNO₃ concentration at these heights. The smaller gradient of the $T_c(\text{NAT})$ curve below 15 km reflects the decrease in nitric acid concentration with decreasing height. The $T_c(\text{NAT})$ curve becomes locally similar to our measured temperature profile at altitudes between ~ 18 and 23 km, with $\Delta T = T - T_c(\text{NAT})$ becoming locally as small as ~ 2 K at around 23 and 20 km. Figure 2 also shows that zonally averaged temperatures²⁶ in December at a latitude of 75°N are markedly higher than T_c . However, minimum temperatures (shown in Fig. 2 for selected pressure levels) measured at the radiosonde station of Sodankylä (230 km east of Esrange) in January and February of 1982, 1983 and 1984 become distinctly lower than $T_c(\text{NAT})$ at altitudes between about 17 km and 24 km and around 13 km. Around the latter height, minimum temperatures become even slightly lower than $T_c(\text{H}_2\text{O-ice})$. Hence, conditions for NAT aerosol formation seems to be favourable in two layers, at 17–24 km and ~ 13 km, whereas H₂O-ice aerosol formation seems to be possible only in the lower layer. It must be kept in mind, however, that these conclusions refer to only a single station (Sodankylä) and a period of only three years (1982, 1983 and 1984). The values of $T_c(\text{NAT})$ and $T_c(\text{H}_2\text{O-ice})$ at 23 km are shown in Fig. 1. Evidently, at the core of the vortex T became markedly lower than $T_c(\text{NAT})$ from day 16 to day 64 (a period of seven weeks). In contrast, at Esrange T became lower than $T_c(\text{NAT})$ at the 30 hPa-pressure level only during a single short period from around day 47 to day 51. With a model $T_c(\text{NAT})$ value of 193 K at the 30-hPa level, the area where NAT aerosol formation may have occurred on 1 February 1988 is relatively large, approximately the size of Greenland.

In conclusion, it seems that the upper limit to $T_c(\text{NAT})$ set by our present data is consistent with model predictions based on our nitric acid measurements, recent laboratory data⁸ on macroscopic NAT mixtures and typical stratospheric abundances for water. The low T_c values mean that NAT aerosol formation may occur in the 'core' region of Arctic vortex but seems to be rare in outer regions of the vortex, at the latitude of Kiruna for example. Hence, the spatial and temporal distribution of NAT aerosol in the Arctic seems to be rather limited in extent compared with the Antarctic stratosphere, where average winter temperatures are about 5–10 K lower. However, lower temperatures may also reduce the probability of NAT formation by inducing heterogeneous condensation of 'pure' water vapour on NAT aerosols followed by rapid gravitational settling of relatively large water-ice particles containing NAT cores. Both nitric acid and water may thereby become depleted in the coldest layer, which would reduce the probability of subsequent NAT aerosol formation at these heights. Besides the larger dynamical activity, the higher temperatures of the Arctic vortex may therefore be a major reason for the absence of pronounced ozone depletion in the Arctic. However, a cooling of the winter polar Arctic vortex by only a few degrees may be sufficient to make NAT aerosol formation much more widespread in the Arctic. Such a cooling could be induced in the future by the rising content of CO₂ and other infrared active gases in the atmosphere, which cools the stratosphere by emission of infrared radiation. Recent climate models²⁷ predict a cooling of the Arctic vortex in winter by up to ~ 6 K. Changes of the stratospheric water vapour abundance, which could result from changes of tropical

tropopause temperatures associated with a troposphere greenhouse effect, would also be important. □

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- Crutzen, P. & Arnold, F. *Nature* **324**, 651–655 (1986).
- Toon, O. B., Hamill, P., Turco, R. P. & Pinto, G. *Geophys. Res. Lett.* **13**, 1284 (1986).
- McElroy, M. B., Salawitch, R. J. & Wofsy, S. C. *Geophys. Res. Lett.* **13**, 1296–1299 (1986).
- Poole, L. R. & McCormick, M. P. *Geophys. Res. Lett.* **15**, 21–23 (1988).
- Molina, M. J., Tso, T. L., Molina, L. T. & Wang, F. C. Y. *Science* **238**, 1253–1257 (1987).
- Tolbert, M. A., Rossi, M. J., Malhorta, R. & Golden, D. *Science* **238**, 1258–1260 (1987).
- Leu, M. T. *Geophys. Res. Lett.* **15**, 17–20 (1988).
- Hanson, D. & Mauersberger, K. *Geophys. Res. Lett.* **15**, 855–858 (1988).
- Arnold, F., Heitmann, H. & Oberfrank, K. *Planet. Space Sci.* **32**, 1567–1576 (1984).
- Arnold, F. & Hauck, G. *Nature* **315**, 307–309 (1985).
- Knop, G. & Arnold, F. *Planet. Space Sci.* **33**, 983–986 (1985).
- Arnold, F., Knop, G. & Ziereis, H. *Nature* **321**, 505–507 (1986).
- Knop, G. & Arnold, F. *Planet. Space Sci.* **35**, 259–266 (1987).
- Arnold, F. & Knop, G. *Int. J. Mass Spectrom. Ion Proc.* **81**, 33–44 (1987).
- Knop, G. & Arnold, F. *Geophys. Res. Lett.* **14**, 1262–1265 (1987).
- Arnold, F. et al. *A. Rep. Max-Planck-Institut für Kernphysik* (1988).
- Gille, J. C. et al. *J. geophys. Res.* **89**, 5179–5190 (1984).
- Gille, J. C. & Russell III, J. M. *J. geophys. Res.* **89**, 5125–5140 (1984).
- Austin, J., Garcia, R. R., Russell, J. M., Solomon, S. & Tuck, A. F. *J. Geophys. Res.* **91**, 5477–5485 (1986).
- Jackman, C. H., Guthrie, P. D. & Kaye, J. A. *J. geophys. Res.* **92**, 995–1008 (1987).
- Girard, A., Gramont, L., Loissard, N., Boiteux, S. & Fergant, G. *Geophys. Res. Lett.* **9**, 135–138 (1982).
- Girard, A. et al. *J. geophys. Res.* **88**, 5377–5392 (1983).
- Murray, D. G., Barker, D. B., Brooks, J. N., Goldman, A. & Williams, W. J. *Geophys. Res. Lett.* **2**, 223–225 (1975).
- Poole, L. R., Osborn, M. T. & Hunt, W. H. *Geophys. Res. Lett.* **15**, 867–870 (1988).
- Atmospheric Ozone 1985*, WMO Report No. 16, Vol. II, 474 (1985).
- US Standard Atmosphere, 1966. U.S. Government Printing Office, Washington D.C. 20402.
- Kiehl, J. T., Boville, B. A. & Priegleb, B. P. *Nature* **332**, 501–504 (1988).

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Increased particle flux to the deep ocean related to monsoons

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MONSOONS cause seasonal reversals in the surface circulation of the northern Indian Ocean¹. In the Arabian Sea this results in the upwelling of nutrient-rich water along the coasts^{2–4}, making it one of the highly productive regions of the world's oceans. To assess the impact of monsoon-driven processes on the downward particle flux variations in the open ocean we deployed three moored arrays consisting of six time-series sediment traps at selected locations in the western, central and eastern parts of the deep northern Arabian Sea. Strong seasonality was recorded in particle flux at all three sites with peaks during the south-west and north-east monsoons. High primary productivity during the monsoons resulting from wind-induced mixed-layer deepening and the associated nutrient injection to the euphotic zone appeared to be the main factor controlling the observed particle flux pattern. These findings may shed light on CO₂ uptake during glaciation when wind speeds were higher.

Three mooring systems, each consisting of two time-series sediment traps⁵—one 1,000 m below the sea surface and the other 1,000 m above the sea bottom—were deployed at three

sites in the western, central and eastern Arabian Sea (Fig. 1). The collecting cups were poisoned with mercuric chloride before deployment. The sediment traps were programmed to measure the flux of sinking particles at intervals of 12 to 13 days each over a duration of six months per deployment. They have been recovered and redeployed three times since May 1986 using the research vessels RV *Sonne* and the ORV *Sagar Kanya*. Here we report the results obtained during the first year of deployment.

On recovery of the traps, the samples were wet-sieved and split using a precision rotary splitter. One quarter of the <1 mm size fraction was filtered through pre-weighed Nuclepore filters (0.5 µm) and dried at 40 °C. This fraction was used for flux calculations and for analysis of first order parameters such as carbonate, opal, lithogenic, organic carbon, and nitrogen^{6,7}.

At all three sites the flux of sinking particles showed seasonality which is strongly related to the monsoons with higher fluxes during the south-west (June to September) and north-east (December to February) monsoons (Fig. 2). Individual fluxes differed by more than two orders of magnitude. Differences between maximum and minimum fluxes during these two periods were highest at the western and eastern sites; these are characterized by their proximity to the upwelling centres along the Somali and the Arabian coasts and the Indian continental shelf, respectively. The similarity in flux patterns not only at sites close to the upwelling centres, but also at the site in the central Arabian sea, indicates that factors other than coastal upwelling might also be responsible for the higher particle fluxes. The close relationship observed between particle fluxes and wind speeds at the three sites provides a clue (Fig. 3). Higher wind speeds lead to a deepening of the mixed layer and the introduction of nutrient-rich subsurface waters into the euphotic zone^{8,9}. Mixed-layer deepening from 30 to 40 m during the pre-monsoon period to more than 100 m during the south-west monsoon have been reported¹⁰. The wind-induced nutrient pumping into the euphotic zone is especially effective in the Arabian Sea as subsurface waters are extremely rich in nutrients¹¹. This results in high primary production during both monsoons^{12–14} and this high 'new production' is reflected in high particle fluxes to the deep sea. An exception to this is the eastern Arabian Sea during the north-east monsoon. Here particle fluxes are extremely low, although wind speeds are only slightly lower than at the other stations. This could be due to the absence of regular winter blooms caused by the inflow of low-salinity water from the eastern tropical Indian Ocean, which leads to stronger stratification and to lower productivity¹³.

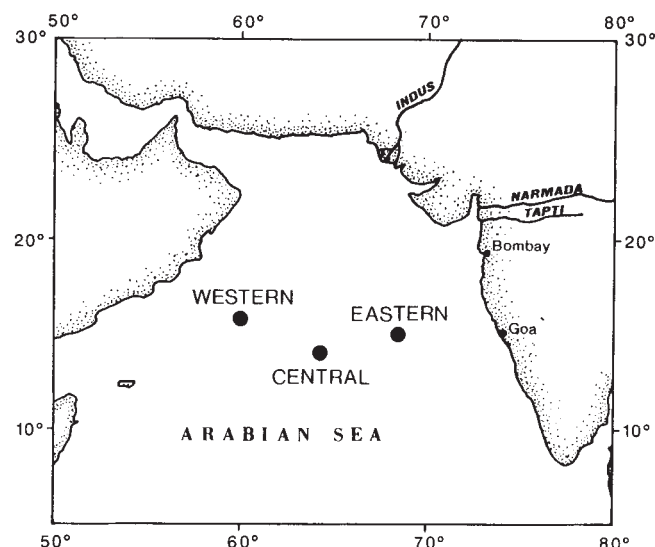
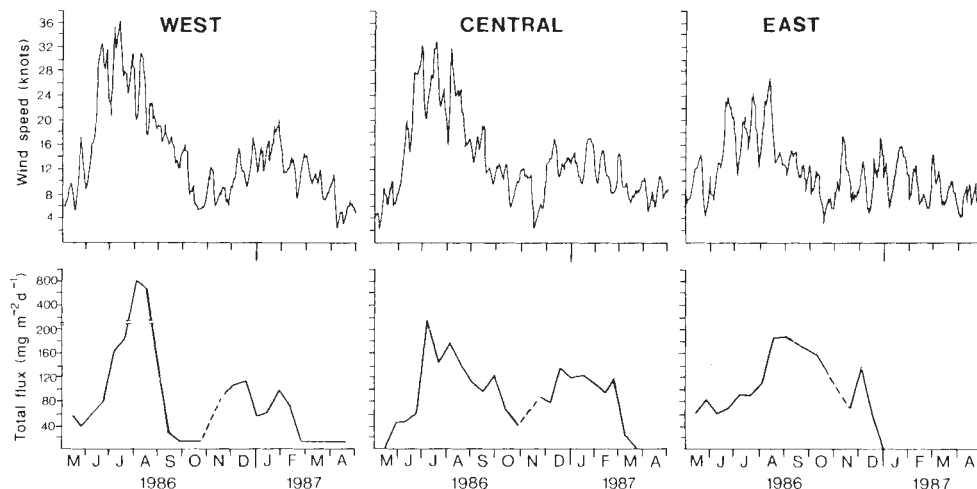


FIG. 1 Locations of mooring systems in the western, central and eastern Arabian Sea. The distance of the moorings from the nearest coast are 520, 930 and 430 km for the eastern, central and western trap, respectively.

FIG. 2 Seasonal variations of particle fluxes to the deep Arabian Sea (bottom) from time-series sediment trap deployments (sampling intervals 12–13 days) at the western, central and eastern Arabian Sea (trap depths: 3,020 m, 2,900 m and 2,770 m, respectively), and wind-speed data during the period of deployment (top) compiled from ship observations (Indian Daily Weather Report, Pune, India) within a radius of 2° around the three sediment trap locations. Wind-speed data were smoothed with a 5-point moving average. Peak fluxes occurring during the SW (June–September) and NE (December–February) monsoons show close similarity to wind-speed patterns and are related to high primary production resulting from wind-induced mixed layer deepening and associated nutrient injection in the euphotic zone^{8,9}, and is best established in the central Arabian Sea (see Fig. 3). The peak fluxes in the western Arabian Sea during July–August and between August–October in the eastern Arabian Sea also have components derived from nutrient advection¹⁶ and lateral transport of material from the coastal



upwelling centres. In the eastern Arabian Sea strong stratification resulting from the inflow of low-salinity water from the eastern tropical Indian Ocean during the NE monsoon leads to low productivity¹³ reflected in low particle fluxes.

Further information on factors controlling the observed fluxes comes from the component fluxes (Table 1). Carbonates dominate the component fluxes and are present mainly in the form of coccolithophorids with a certain amount of foraminifera. Organic carbon and nitrogen fluxes are similar to those reported from other oceanic areas¹⁵. Major differences between sites were observed in the opal and lithogenic fluxes.

In the central and eastern Arabian Sea sites, opal accounts for 12% and 13% of the total flux. In the western Arabian Sea, opal contributed up to 22% of the total annual flux and accounted for as much as 40% during the peak fluxes in July and August, mostly in the form of the diatom *Rhizosolenia*, a major upwelling species. During the summer monsoon of 1986, waters from the Somali-Arabian upwelling centres have been shown to be advected up to 60° E (ref. 16). Peak fluxes at this site located more than 400 km from the continental margin thus have a component derived from nutrient-rich upwelled water transported offshore.

High lithogenic fluxes are associated with the south-west monsoon (June to September) and their contribution to total fluxes increases from the western (9%) to the eastern (25%)

Arabian Sea site (Table 1). Eolian input to the Arabian Sea is restricted by the Somali Jet to the northwestern parts, and may constitute an important source of lithogenic particles at the western Arabian Sea site (ref. 17; F. Sirocco and M. Sarnthein, manuscript in preparation). In the northeastern and central parts of the Arabian Sea the chief suppliers of lithogenic material are the rivers Indus, Narmada and Tapti. More than 80% of their annual suspended-matter discharge occurs during the southwest monsoon¹⁸. The simultaneous prevalence of clockwise surface currents in the Arabian Sea can divert lithogenic material to the eastern Arabian Sea, contributing to its relative enrichment. Sediment-trap studies in the outer shelf off Goa, India, have shown Indus-derived sediment to be a significant component during this period¹⁹.

Our results show that the particle fluxes out of the surface layers in the Arabian Sea are primarily linked to changes in the prevailing meteorological and hydrographical conditions through biological processes occurring in the surface layers. Higher wind speeds promote biological productivity by (1) enhancing the introduction of essential trace elements associated with atmospheric dust fallout (for instance, iron²⁰), and (2) the

TABLE 1 Component fluxes of the fraction <1 mm to the deep Arabian Sea. The data are given for the SW (June–September), NE (December–February) monsoons and for the periods in between

Site	Components <1 mm	NE–SW		SW Monsoon		SW–NE		NE monsoon		Total	
		g m ⁻²	%	g m ⁻²	%	g m ⁻²	%	g m ⁻²	%	g m ⁻²	%
Western Arabian Sea	carbonate	1.17	54.2	12.10	51.4	2.13	73.2	3.60	68.1	19.00	56.00
	opal	0.12	5.6	6.08	25.8	0.30	10.3	0.78	14.7	7.28	21.5
	lithogenic	0.11	5.1	2.10	8.9	0.28	9.6	0.15	2.8	2.64	7.8
	C _{org}	0.16	7.4	1.18	5.0	0.14	4.8	0.32	6.0	1.80	5.3
	N	0.02	0.9	0.14	0.6	0.02	0.7	0.04	0.8	0.22	0.6
	Total flux	2.16	6.4	23.56	69.5	2.91	8.6	5.29	15.6	33.92	
Central Arabian Sea	carbonate	0.49	57.6	9.31	66.4	2.15	66.8	5.55	66.4	17.50	66.2
	opal	0.06	7.1	1.51	10.8	0.35	10.9	1.16	13.9	3.08	11.6
	lithogenic	0.07	8.2	1.84	13.1	0.35	10.9	0.79	9.5	3.05	11.5
	C _{org}	0.06	7.1	0.74	5.3	0.20	6.2	0.53	6.3	1.53	5.8
	N	0.01	1.2	0.09	0.6	0.02	0.6	0.07	0.8	0.19	0.7
	Total flux	0.85	3.2	14.02	53.0	3.22	12.2	8.36	31.6	26.45	
Eastern Arabian Sea	carbonate	0.80	43.2	7.27	49.5	3.40	54.4	0.41	51.3	11.88	50.4
	opal	0.30	16.2	1.81	12.3	0.79	12.6	0.23	28.8	3.13	13.3
	lithogenic	0.42	22.7	3.60	24.5	1.35	21.6	0.03	3.8	5.40	22.9
	C _{org}	0.18	9.7	1.00	6.8	0.33	5.3	0.05	6.3	1.56	6.6
	N	0.02	1.1	0.10	0.7	0.04	0.6	0.01	1.2	0.17	0.7
	Total flux	1.85	7.8	14.68	62.3	6.25	26.5	0.80	3.4	23.58	

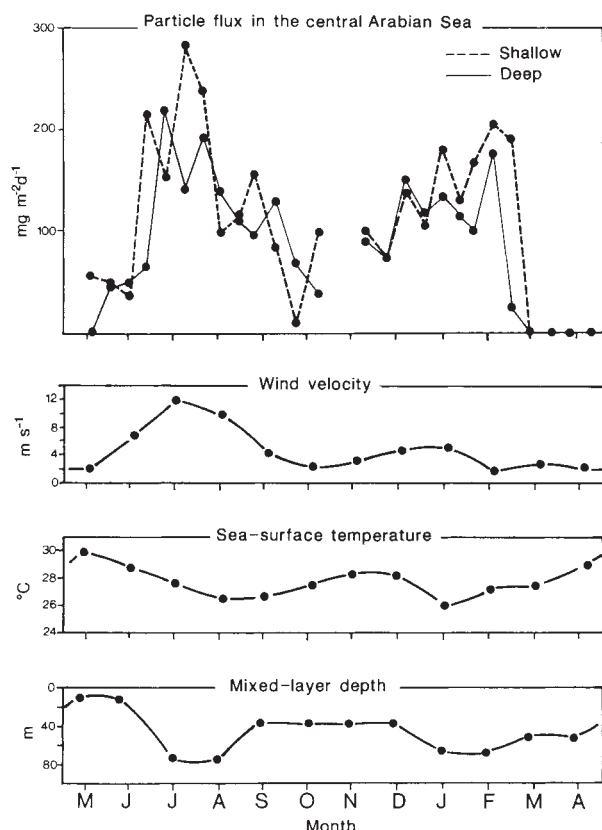


FIG. 3 Time-series (sampling intervals 12–13 days) of particle flux from two depths (shallow, 780 m; deep, 2,900 m) in the central Arabian Sea, and the general information on wind velocity²³, sea-surface temperature¹ and mixed layer depth¹. Low sea-surface temperatures and a deeper mixed layer during these periods show that wind-induced mixing occurs which brings nutrient-rich water to the surface. The resultant high biological productivity leads to the observed flux pattern.

injection of new nutrients from deeper layers to the euphotic zone. There have been times in the recent geological past when wind speeds were higher than today's by a factor of 1.3 to 1.6, and the atmosphere had higher dust contents, for example during the glacial periods²¹, which must have led to an increase in global ocean productivity. The subsequent removal of this new production to the deep ocean as deduced from our study could have contributed in part to low atmospheric CO₂ contents during the glacial periods²². □

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- Wyrki, K. *Oceanographic Atlas of the International Indian Ocean Expedition* (National Science Foundation, Washington, 1971).
- Bruce, J. G. *J. mar. Res.* **32**, 419–423 (1974).
- Currie, R. I., Fisher, A. E. & Hargreaves, P. M. in *The Biology of the Indian Ocean* (eds Zeitschel, B. & Gerlach, S. A.) 37–52 (Springer, Berlin, 1973).
- Sharma, G. S. *Indian J. Mar. Sci.* **7**, 209–218 (1976).
- Honjo, S. & Doherty, K. W. *Deep Sea Res.* **35**, 133–149 (1988).
- Honjo, S., Manganini, S. J. & Cole, J. J. *Deep Sea Res.* **29**, 609–625 (1982).
- Michaelis, W. & Ittekkot, V. *Transport of Carbon and Minerals in World Rivers Part 1* (ed Degens, E. T.) 233–243 (Mitt. Geol.-Pal. Inst., Univ. Hamburg, SCOPE/UNEP Sonderbd. 52, 1982).
- Klein, P. & Coste, B. *Deep Sea Res.* **31**, 21–37 (1984).
- Kiefer, D. A. & Kremer, J. N. *Deep Sea Res.* **28A**, 1087–1105 (1981).
- Sastry, J. S. & Ramesh Babu, V. *Proc. Indian Acad. Sci. (Earth planet. Sci.)* **94**, 117–128 (1985).
- Ryther, J. H. & Menzel, D. W. *Deep Sea Res.* **12**, 199–209 (1965).
- Banse, K. *Deep Sea Res.* **34**, 713–723 (1987).
- Banse, K. & McClain, C. R. *Mar. Ecol. Prog. Ser.* **34**, 201–211 (1986).
- Qasim, S. Z. *Deep Sea Res.* **29**, 1041–1068 (1982).
- Tsunogai, S. & Noriki, S. *Deep Sea Res.* **34**, 755–767 (1987).
- Vinayachandran, P. N., Sadhuram, Y. & Ramesh Babu, V. *Tellus* (submitted).
- Savoie, D. L., Prospero, J. M. & Nees, R. T. *J. geophys. Res.* **92**, 933–942 (1987).
- Ittekkot, V. & Arain, R. *Geochim. cosmochim. Acta* **50**, 1643–1653 (1986).
- Ramaswamy, V. in *Particle Flux in the Ocean* (eds Degens, E. T., Honjo, S. & Izard, E.) 233–243 (Mitt. Geol.-Pal. Inst., Univ. Hamburg, SCOPE/UNEP Sonderbd. 62, 1987).
- Martin, J. H. & Fitzwater, S. E. *Nature* **331**, 341–343 (1988).
- Petit, J. R., Briat, M. & Royer, A. *Nature* **293**, 391–394 (1981).

- Barnola, J. M., Raynaud, D., Korotkevich, Y. S. & Lorius, C. *Nature* **329**, 408–414 (1987).
- Ramage, G., Miller, F. R. & Jefferies, C. *International Indian Ocean Expedition Atlas 1: the surface climate of 1963 and 1964* (East West Center, Honolulu, 1969).

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Barium content of benthic foraminifera controlled by bottom-water composition

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THE carbon isotope ratio ($\delta^{13}\text{C}$) and cadmium content (Cd/Ca) of benthic foraminifera shells have been used to reconstruct deep-water circulation patterns of the glacial oceans^{1–7}. These tracers co-vary with phosphorus in the modern ocean because they are nearly quantitatively regenerated from sinking biological debris in the upper water column. Hence they can be used to reconstruct the distribution of labile nutrients in glacial water masses. Independent constraints on glacial deep-ocean circulation patterns could be provided by a tracer of the distribution of silica and alkalinity, the deeply regenerated constituents of planktonic hard parts. Barium shares key aspects of its behaviour with these refractory nutrients because it is removed from solution in surface waters and incorporated into sinking particles which slowly dissolve deep in the water column and in the sediments⁸. The fractionation of Ba between deep-water masses of the major ocean basins is largely controlled by thermohaline circulation patterns, so Ba conforms to different boundary conditions from Cd and $\delta^{13}\text{C}$. As Ba substitutes into trigonal carbonates⁹, it is a potential palaeoceanographic tracer if the Ba content of foraminifera shells reflects ambient dissolved Ba concentrations. Here we present data from Recent core-top benthic foraminifera which indicate that the Ba content of some recent calcitic benthic foraminifera does co-vary with bottom-water Ba.

The distribution of barium, alkalinity and silica in the world's oceans was mapped extensively during the GEOSECS

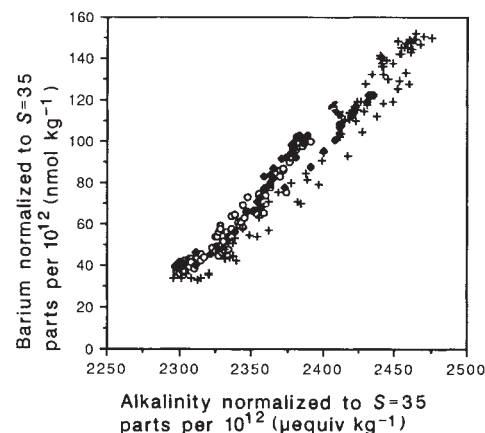


FIG. 1 Paired Ba and alkalinity data from ocean water samples analysed for the GEOSECS program^{10–13}. Alkalinity and barium are normalized to a constant salinity of 35‰. Included are: Atlantic Ocean stations 29, 82 and 111 (circles); Pacific Ocean stations 204, 226, 312 and 322 (crosses); and Indian Ocean stations 429 and 452 (diamonds).

program¹⁰⁻¹³. Figure 1 illustrates the similarity between Ba and alkalinity over nine widely spaced GEOSECS sites. Although the cycling of these two tracers is dominated by different mechanisms^{14,15}, the overprint of thermohaline circulation on two dissolved constituents sharing similar sites of uptake and regeneration results in strong covariance throughout the world's oceans. Thus the distribution of Ba in glacial oceans can provide direct insight into the distribution of refractory nutrients such as alkalinity.

To calibrate the response of benthic foraminifera shells to ambient Ba concentrations we have measured Ba in foraminifera recovered from Recent core tops lying below bottom waters whose Ba concentrations can be estimated from nearby GEOSECS stations. Foraminifera were hand-picked from disaggregated sediment samples and cleaned with a series of physical and chemical steps designed to remove any extraneous Ba not substituted into the lattice of the foraminiferal CaCO_3 . These steps begin with the foraminiferal Cd cleaning method⁶, followed by dissolution of associated sedimentary barite (BaSO_4) using alkaline diethylenetriamine-pentaacetic acid (DTPA)¹⁶. The typical sample mass before cleaning is 0.6 mg (10–30 individuals). Barium concentrations are quantified by isotope-dilution flow-injection analysis on an inductively coupled

plasma-mass spectrometer (ICP-MS). Samples are spiked with ^{135}Ba and the $^{135}\text{Ba}/^{138}\text{Ba}$ ratio is measured subsequently. Accuracy is based on calibration of isotope-dilution determinations to a gravimetric standard. The detection limit of the flow-injection method is less than 0.2 nmol l^{-1} for a 200- μl injection volume (5 pg Ba). Signal-to-noise ratios of samples were generally greater than 50:1. Calcium is quantified by flame atomic absorption. The inter-run reproducibility for 39 analyses of a consistency standard containing 9.6 nmol l^{-1} Ba and 4.1 mmol l^{-1} Ca ($\text{Ba}/\text{Ca} = 2.3 \text{ } \mu\text{mol mol}^{-1}$) was 2.6% for Ba, 1.5% for Ca and 3.1% for the Ba/Ca ratio. Although contamination during sample preparation and handling is negligible, occasional individual analyses are unreliable because of insufficient cleaning.

The Ba/Ca ratio of the three species studied (Fig. 2a, b and c; Table 1) increases linearly with increasing concentration in the bottom water. The scatter in the plots exceeds analytical precision and uncertainties in estimating Ba from nearby stations. This scatter may result either from bioturbation, which can mix older individuals (which may have lived at times when bottom-water Ba concentrations were different) into the core top, or from a limit on the reliability of certain species of foraminifera as recorders of ambient Ba concentrations. We

TABLE 1 Ba/Ca ratios in benthic foraminifera from Recent core tops

Core	Sample depth (cm)	Latitude	Longitude	Water depth (m)	Estimated bottom-water Ba (nmol kg ⁻¹)	Ba/Ca (μmol mol ⁻¹)		
						<i>C. wuellerstorfi</i>	<i>C. kullenbergi</i>	<i>Uvigerina</i> spp.
AI154 5PG	2–4	07°25' S	89°10' W	4,165	133	4.18		4.16
AI107 65GGC	4–7	32°02' S	36°11' W	2,795	64	2.15		
AI107 67GGC	0–2	31°55' S	36°12' W	2,587	63	1.89 ± 0.06 (<i>n</i> = 2)		
AI107 69GGC	5–7	31°40' S	36°01' W	2,158	66			2.26
AI107 70GGC	2–4	31°36' S	35°59' W	2,079	66	2.20		2.04
AI107 71GGC	0–2	31°31' S	35°56' W	1,887	68	2.24		2.00
CHN82 1PC	2–4	36°06' N	07°10' S	830	47			2.18
CHN82 3PC	13–16	41°38' N	27°20' W	2,525	51	1.77		
CHN82 4PC*	0–2	41°43' N	32°51' W	3,427	56	2.44	2.26 ± 0.01 (<i>n</i> = 2)	
CHN82 9PC	7–10	41°51' N	26°27' W	2,830	52	1.85		
CHN82 11PC*	1–6	42°23' N	31°48' W	3,209	55	2.25	2.18 ± 0.06 (<i>n</i> = 2)	
CHN82 15PC	3–5	43°14' N	28°08' W	2,155	50	1.98 ± 0.02 (<i>n</i> = 3)		
CHN82 20PG*	4–7	43°30' N	29°52' W	3,070	53	1.82	2.63 ± 0.16 (<i>n</i> = 2)	
CHN82 21PG	4–8	43°17' N	29°50' W	2,103	49	1.92	2.52 ± 0.04 (<i>n</i> = 2)	
EN66 10GGC	1–2	06°39' N	21°54' W	3,527	74	2.58	2.67	
EN66 16GGC	1–3	05°28' N	21°08' W	3,152	73	2.79	2.28	
EN66 21GGC*	3–12	04°14' N	20°38' W	3,995	75	2.75	2.87	
EN66 26GGC	1–3	03°05' N	20°01' W	4,745	77	2.86	2.35	
EN66 32GGC*	0–3	02°28' N	19°44' W	5,003	77	3.00		
EN66 36GGC	2–3	04°19' N	20°13' W	4,270	75	2.45		
EN66 38GGC	2–4	04°55' N	20°30' W	2,931	71	2.31	2.39	2.56
EN66 44GGC	1–2	05°16' N	21°43' W	3,428	73	2.89	2.72	
IOS82 PCS01	2–4	42°23' N	23°31' W	3,540	61		2.54	
KNR64 5PG	6–8	16°32' N	74°48' W	3,047	56	2.26	2.42	
KNR73 3PC*	5–6	00°22' S	106°11' W	3,606	134	4.95		
OC173 G	4–6	31°54' N	64°18' W	4,469	65	2.16 ± 0.10 (<i>n</i> = 3)		
PS21295–4*	1–2	78°00' N	02°25' E	3,112	46	2.03		
RC11–120*	5–7	43°31' S	79°52' E	3,135	91	3.41		
TR163–14	3–5	05°41' N	87°14' W	2,365	132	4.12		
TR163–27	2–3	02°15' S	86°35' W	3,180	135			4.66
TR163–28	1–2	29°19' S	86°14' W	3,200	135			4.09
TR163–31B*	1–11	03°57' S	85°58' W	3,210	133	4.41 ± 0.24 (<i>n</i> = 3)		
TR163–32	2–4	02°29' S	82°59' W	2,890	134		4.50	4.02 ± 0.47
V18–68*	10–12	54°33' S	77°51' W	3,972	110	3.43	5.04	3.93 ± 0.15 (<i>n</i> = 2)
V22–174*	9–11	10°04' S	12°49' W	2,630	76			3.94
V22–197*	14–17	14°10' N	18°35' W	3,167	72	3.10		1.94
V22–198	4–6	14°35' N	19°40' W	1,082	62		2.52	2.80
V24–109	5–8	00°26' N	158°48' E	2,367	136	3.76		
V27–60	5–8	72°11' N	08°34' E	2,525	49	2.11		
V27–86*	5–8	66°36' N	01°07' E	2,900	51	1.90		
V28–56*	3–4	68°02' N	06°07' W	2,941	51	1.97		
V28–304	6–9	28°32' N	134°08' E	2,942	139	3.69		
V32–159	15–17	48°40' N	147°24' E	1,235	109			2.94

* Cores with >15 cm Holocene sediment

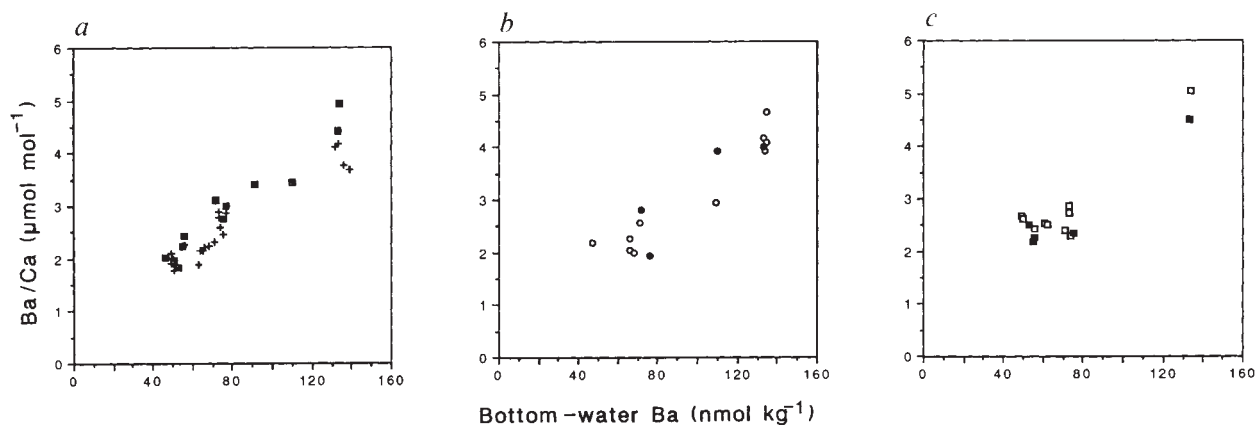


FIG. 2 Ba/Ca in recent benthic foraminifera plotted against estimated bottom-water barium concentrations. Samples from cores with >15 cm of

Holocene sediment are indicated by darkened symbols. *a*, *C. wuellerstorfi*; *b*, *Uvigerina* spp.; *c*, *C. kullenbergi*.

believe the first factor to be a primary cause of scatter for *C. wuellerstorfi* (Fig. 2a) because the most deviant points (low Ba/Ca ratios for high-Ba Pacific points) are from cores with lower sedimentation rates. Because data for the other two species are more limited, we can not clearly identify the cause of the offsets. Of the three species, *C. wuellerstorfi* shows the least scatter. However, we cannot conclude that this species is necessarily a more reliable recorder of bottom-water Ba because bioturbation combined with temporal abundance variations can bias core tops such that the mean age of each species present might not be the same. Because *Uvigerina* spp. is generally low in abundance during interglacials, *Uvigerina* spp. recovered from core tops are probably less likely to be representative of recent conditions than are *C. wuellerstorfi*.

We average the data in Fig. 2 to calculate an effective distribution coefficient $D = (\text{Ba/Ca})_{\text{foraminifera}} / (\text{Ba/Ca})_{\text{sea water}}$. For the fractionation of Ba in the calcite of the three benthic species, $D = 0.37 \pm 0.06$. In the present data set there is no evidence for significant differences in individual D values for each species, although mean D values range from 0.34 for *Uvigerina* spp. and 0.37 for *C. wuellerstorfi* to 0.41 for *C. kullenbergi*. The uncertainty in D for each species can be improved by further analyses from high-sedimentation-rate cores, ultimately allowing us to determine whether different benthic species have different effective distribution coefficients.

The estimate of the foraminiferal distribution coefficient applies only to cold deep waters (temperature $\sim 3^\circ\text{C}$, pressure ~ 300 bar). Inorganic-precipitation experiments performed at 20–25°C and 1 bar pressure generally yield distribution coefficients of the order of 0.1, although much higher values are observed during the first stages of precipitation or during rapid precipitation⁹. Observed foraminiferal D_{Sr} for chemically similar Sr is about a factor of four times higher than that observed for slow precipitation^{17–19}. Measurements of Ba in planktonic foraminifera indicate an effective distribution coefficient of about 0.2 (ref. 20), although the restricted range of Ba concentrations in surface waters limits validation of the direct response of planktonic foraminifera to changes in surface-water Ba concentrations. That this value is significantly different from that for benthic foraminifera suggests that variables such as temperature, pressure and biological factors may play a role in determining D .

Thus it will be possible to use fossil shells from deep-sea cores to reconstruct the distribution of Ba in the bottom waters of past oceans. A preliminary examination of cores from the North Atlantic indicates that benthic foraminifera recovered from glacial sections have up to 50% higher Ba/Ca ratios than are observed for the Holocene individuals. Higher glacial Ba in the deep Atlantic is an indication of reduced flushing of the Atlantic by nutrient-depleted waters during glacial periods. □

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- Curry, W. B., Duplessy, J.-C., Labeyrie, L. D. & Shackleton, N. J. *Paleoceanography* **3**, 317–342 (1988).
- Duplessy, J.-C. *et al.* *Paleoceanography* **3**, 343–360 (1988).
- Sarnthein, M., Winn, K., Duplessy, J.-D. & Fontugne, M. R. *Paleoceanography* **3**, 361–399 (1988).
- Keigwin, L. D. *Nature* **300**, 362–364 (1987).
- Boyle, E. A. & Keigwin, L. D. *Nature* **300**, 35–40 (1987).
- Boyle, E. A. & Keigwin, L. D. *Earth planet. Sci. Lett.* **76**, 135–150 (1985).
- Boyle, E. A. & Keigwin, L. D. *Science* **218**, 784–787 (1982).
- Chan, L. H., Drummond, D., Edmond, J. M. & Grant, B. *Deep Sea Res.* **24**, 613–649 (1977).
- Kitano, Y., Kanamori, N. & Oomori, T. *Geochem. J.* **4**, 183–206 (1971).
- Bainbridge, A. E. *GEOSECS Atlantic Expedition Vol. 1, Hydrographic Data*, (NSF, Washington, DC, 1981).
- Broecker, W. S., Spencer, D. W. & Craig, H. *GEOSECS Pacific Expedition Vol. 3, Hydrographic Data* (NSF, Washington, DC, 1982).
- Weiss, R. F., Broecker, W. S., Craig, H. & Spencer, D. W. *GEOSECS Indian Ocean Expedition Vol. 5, Hydrographic Data* (NSF, Washington, DC, 1983).
- Ostlund, H. G., Craig, H., Broecker, W. S. & Spencer, D. W. *GEOSECS Atlantic, Pacific, and Indian Ocean Expeditions Vol. 7, Shorebased Data and Graphics* (NSF, Washington, DC, 1987).
- Bishop, J. K. B. *Nature* **332**, 341–343 (1988).
- Edmond, J. M. *Deep Sea Res.* **21**, 455–480 (1974).
- Sill, C. W. & Willis, C. P. *Analyt. Chem.* **36**, 622–630 (1964).
- Bender, M. L., Lorens, R. B. & Williams, D. F. *Micropaleontology* **21**, 448–459 (1975).
- Delaney, M. L., Bé, A. W. H. & Boyle, E. A. *Geochim. cosmochim. Acta* **49**, 1327–1341 (1985).
- Lorens, R. B. *Geochim. cosmochim. Acta* **45**, 553–561 (1981).
- Lea, D. W. & Boyle, E. A. *Eos* **68**, 1331 (1987).

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Stress dependence of the mechanism of the olivine–spinel transformation

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OLIVINE, $\alpha\text{-(Mg, Fe)}_2\text{SiO}_4$, is the most abundant phase in the Earth's upper mantle. It transforms to high-density polymorphs (γ , with the spinel structure and β , with a modified spinel structure) under pressure and temperature conditions appropriate to explain the discontinuity in seismic velocities that defines the base of the upper mantle at 400 km depth; the transformation is generally believed to be responsible for that discontinuity¹. Knowledge of the mechanism and kinetics of the transformation is important for understanding certain aspects of mantle dynamics, and extensive experimental and theoretical work has been conducted on both natural olivine and several analogous chemical systems. Previously, conflicting results have suggested a reconstructive transformation mechanism^{2–10}, a martensitic mechanism^{11,12} and a mechanism that is not entirely consistent with either^{13–16}. The conflicts cannot be explained as different mechanisms operating in different systems¹⁷. Here we show that the transformation can proceed by two

different mechanisms, depending on the level of nonhydrostatic stress; under hydrostatic pressure or low stresses (at sufficiently high temperatures), nucleation occurs by an incoherent, diffusion-dependent, intercrystalline mechanism proposed by Sung and Burns^{2,3} and first demonstrated by Vaughan *et al.*⁴ (Fig. 1), whereas under high stresses, nucleation of the high-pressure (γ) polymorph (spinel) occurs by a coherent, shear-induced, intracrystalline mechanism predicted by Poirier¹⁸. Thus we are able to reconcile the previously conflicting results, and consideration of the stress levels likely in natural situations leads us to conclude that both mechanisms are geologically relevant.

As part of a continuing study of the effect of stress on the olivine-spinel transformation¹⁹⁻²¹, we deformed specimens of a synthetic Mg_2GeO_4 olivine polycrystal (an analogue of the mantle material) at a strain rate of 10^{-4} s^{-1} , a temperature of 900 K and pressures of 1.1–1.4 GPa in a modified Griggs apparatus²². These conditions are in the spinel stability field, a minimum of 400 K below the equilibrium phase boundary. The specimens are extremely strong, displaying a flow stress ($\sigma_1 - \sigma_3$) of 1.9 GPa or more in all cases (Table 1).

Optical microscopy of sections of specimens quenched immediately after deformation showed extremely inhomogeneous deformation within individual olivine crystals, but martensitic (stress-induced) lamellae of the high-pressure polymorph were not found. Examination by transmission electron microscopy (TEM), however, revealed (Fig. 2) thin (1–8 nm) lamellae parallel to (100). (To avoid confusion, we index Mg_2GeO_4 olivine according to the unit cell for silicate olivine, in which $b > c > a$.) Selected-area electron diffraction (SAED) patterns showed pronounced streaking parallel to the (100) reciprocal lattice direction, but no diffraction spots attributable to the spinel phase were found (Fig. 2, insert), even at specimen strains approaching 35% shortening. The streaking in the diffraction patterns is attributable to the thin lamellae. X-ray diffraction analysis of powders prepared from these specimens also failed to show any spinel lines.

Deformed specimens annealed at 1200 K (at pressure) for longer than 15 min yielded optically visible, faceted spinel crystals; after 1 hour, spinel crystals as large as 80 μm developed, the average diameter being 50 μm (Fig. 3a). The spinel grains nucleated within the olivine crystals, in contrast to spinels grown at higher temperature under hydrostatic or low-stress conditions, which nucleate exclusively at grain boundaries. The interfacial angles between crystal facets indicate that the spinel habit is {111}; growth of several crystals until they interfere commonly leads to irregular shapes, but the {111} facets remain visible (Fig. 3a). Within a single olivine crystal, one {111} facet of each spinel crystal is parallel to one facet of the other spinels and to (100) of the host olivine. Examination of annealed specimens by TEM (Fig. 3b) revealed coarser lamellae than those present in unannealed specimens, and also larger spinel crystals which had lost the lamellar habit. SAED patterns of regions containing coarser lamellae retained the streaking of the as-deformed material, but displayed extra spots characteristic of spinel. Indexing of the patterns showed that $[100]_{\text{ol}} = [111]_{\text{sp}}$ and $[001]_{\text{ol}} = [\bar{1}10]_{\text{sp}}$. The larger crystal of Fig. 3b, although having lost its lamellar habit, retained its largest dimension parallel to

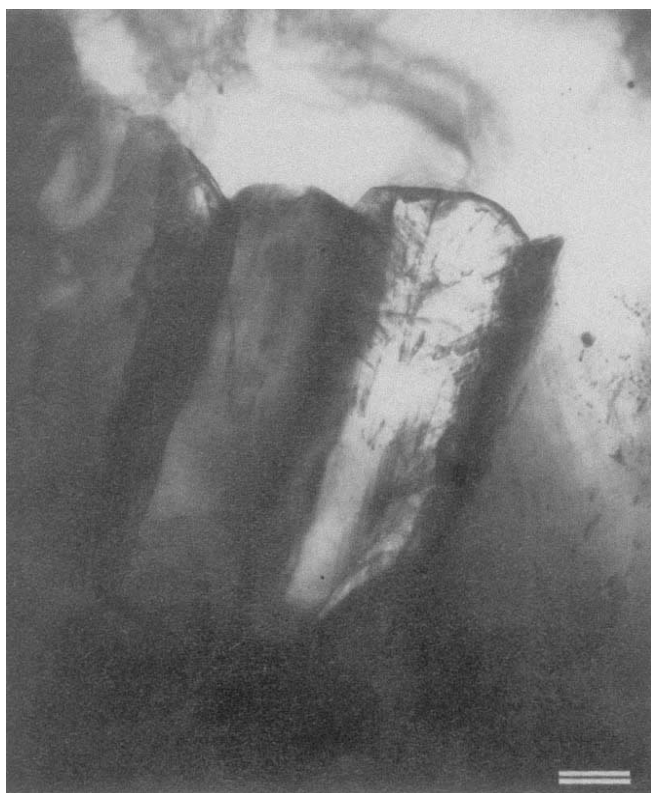


FIG. 1 High-voltage (1,000 kV) transmission electron micrograph of several elongated spinel (γ) crystals growing into a single olivine (α) crystal (white). The variation of contrast and orientation of the planar and linear defects in the spinels demonstrates their widely differing orientations. Electron diffraction confirmed these differences^{4,9}, and showed that none of the crystals exhibit the topotaxy predicted by Poirier¹⁸. Scale bar, 1 μm .

(100)_{ol} and exhibited the same topotaxy as the lamellae. By contrast, a specimen annealed under the same conditions but not previously deformed developed no lamellae and no spinel crystals. Our observation of loss of the lamellar habit as coarsening begins suggests that crystal growth occurs by normal phase-boundary migration, leading to euhedral crystals bounded by {111} faces.

The lamellae have all of the characteristics predicted by the martensitic mechanism of Poirier¹⁸, who constructed his model of the transformation mechanism by analogy with similar martensitic transformations in other systems. In those systems, softening of the appropriate elastic stiffness moduli is observed in the vicinity of the phase boundary. Accordingly, Poirier¹⁸ predicted that the elastic stiffness coefficients C_{55} and C_{66} of olivine should show similar premonitory phenomena. A search for such shear-mode softening in Fe_2SiO_4 was unsuccessful²³. We propose that failure of C_{55} and C_{66} to soften is complementary to our observations, first that a very high stress is necessary for nucleation by the martensitic mechanism, and second that growth of the γ phase under hydrostatic pressure occurs by

TABLE 1 Experimental results

T (K)	P (GPa)	$\dot{\epsilon}$ (s^{-1})	Maximum stress (GPa)	Total strain (%)	Length of 1,200-K anneal (min)	Spinel visible optically	Lamellae visible in TEM	Sample number
902	1.4	1.4×10^{-4}	2.5	23	0	no	yes	GL294
897	1.3	3.2×10^{-4}	1.9	34	2	no	yes	GL306
897	1.1	2.4×10^{-4}	2.2	12	18	yes	yes	GL309
898	1.3	3.4×10^{-4}	2.7	41	58	yes	yes	GL307
1,391	1.7	2.4×10^{-5}	0.2	10	—	yes	no	GL264

$\dot{\epsilon}$ is strain rate.

normal phase-boundary migration rather than by invasion of the crystal by large numbers of partial dislocations.

These new microstructural results not only indicate that there are two separate mechanisms which operate under different conditions, but also provide the information necessary to reconcile the differences between previous studies¹⁷. In particular, our new results lead to an understanding of why low-temperature experiments using synchrotron radiation^{15,16} showed lack of cation ordering in the early stages of the transformation, even though they indicated volume contraction simultaneous with oxygen restacking¹⁷. The answer is inherent in Poirier's model. Briefly, Poirier¹⁸ proposed that under conditions appropriate for the transformation, olivine dislocations with a $[001]$ Burgers' vector gliding on the (100) plane separate into four partial dislocations according to the reaction:

$$[001] \rightarrow \frac{1}{12} [013] + \frac{1}{12} [0\bar{1}3] + \frac{1}{12} [013] + \frac{1}{12} [0\bar{1}3]$$

Implicit in this dislocation mechanism is the fact that a spinel layer produced by passage of the first partial dislocation will have an antiphase relationship with respect to a spinel layer produced by passage of the third partial dislocation (see also Hornstra²⁴). Because the individual lamellae are thin and closely

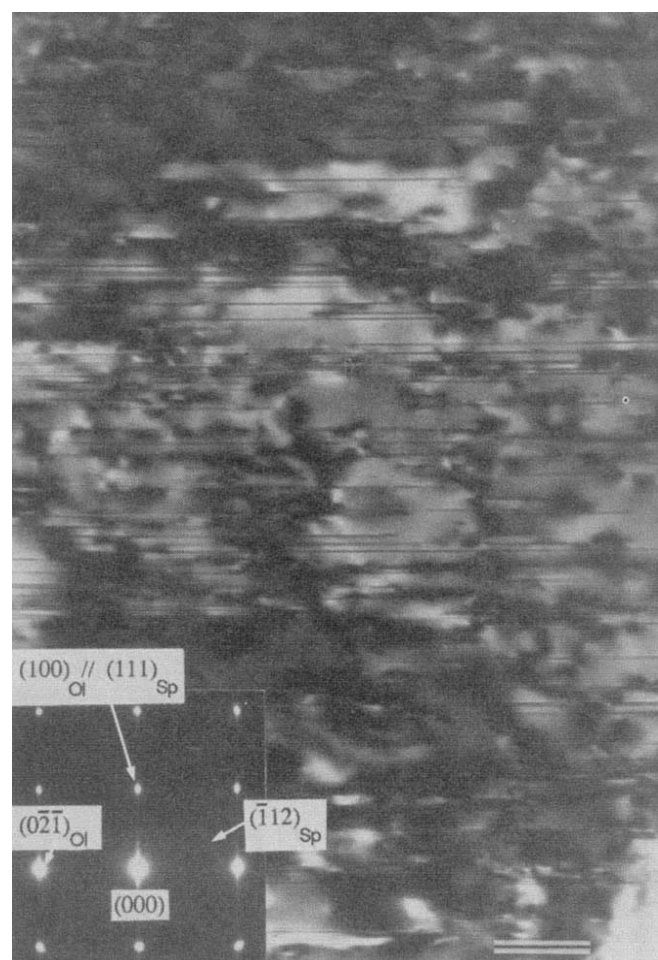


FIG. 2 Transmission electron micrograph and selected-area electron diffraction pattern, suggesting a martensitic nucleation mechanism for the $\alpha \rightarrow \gamma$ phase transformation. The micrograph is viewed parallel to $[0\bar{1}2]_{Ol}$. Thin lamellar features are parallel to (100) . No structure of this type in olivine has previously been seen by the authors or reported in the literature, except for Fig. 1 of ref. 12. Some lamellae can be seen to end. Fuzzy black areas are unit dislocations in poor contrast. The high density of slip dislocations makes clear imaging of the lamellae difficult. The insert in the lower left corner is a selected-area electron diffraction pattern, showing streaking normal to $(100)_{Ol}$. Spots corresponding to spinel (e.g. $[112]_{Sp}$) are not present (arrow). Scale bar, $0.1 \mu\text{m}$.

spaced compared with the minimum dimension resolvable by X-ray diffraction, therefore within the same crystal, groups of lamellae with antiphase relationships will be summed by synchrotron radiation, giving rise to anomalously low apparent structure factors for reflections other than those from the oxygen sublattice. As individual crystal domains grow to dimensions resolvable by X-ray diffraction, so too will grow the intensities of the cation-related peaks. In other words, the synchrotron results have been interpreted correctly in that they require cation disorder on the scale of resolution of the technique ($\sim 100 \text{ nm}$), whereas in rejecting Poirier's mechanism, the implication is that cation disorder is required on the scale of Poirier's model ($\sim 1 \text{ nm}$).

These results also clarify a number of other confusions in the literature. Hamaya and Akimoto^{13,14} grew large, euhedral Ni_2SiO_4 spinels within olivine single crystals. Some of their

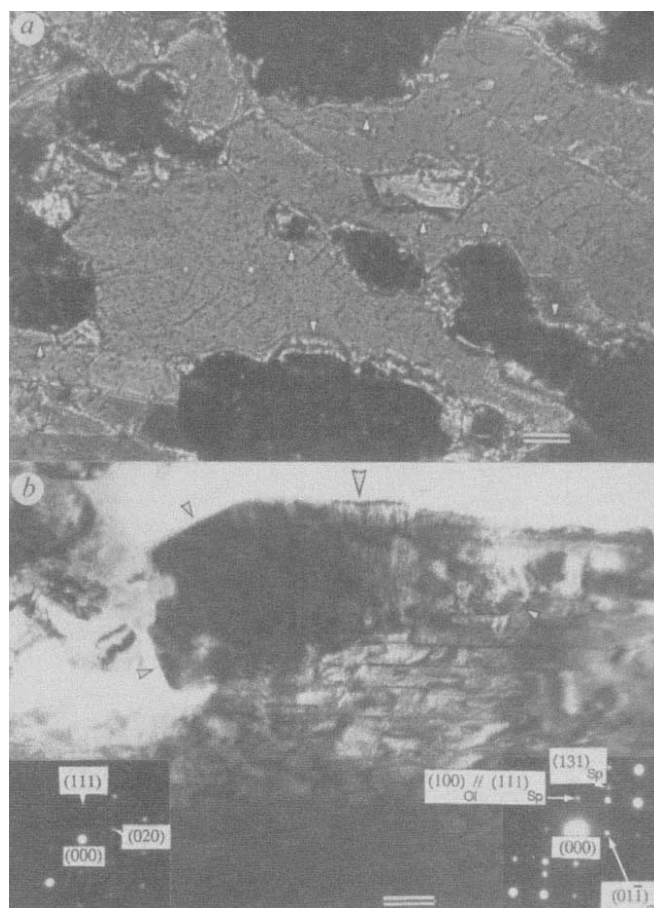


FIG. 3 Annealing at $1,200 \text{ K}$ of specimens deformed at 900 K results in growth of large spinel crystals in the olivine matrix. *a*, Photomicrograph taken between crossed polarizers, showing growth of multiple spinel crystals (isotropic) within a single large olivine grain. The spinels have grown together in many cases, resulting in irregular masses, but each shows a single $\{111\}$ face (indicated by arrowheads) parallel to that of the other spinels and parallel to the trace of (100) of olivine (E-W direction of micrograph). *b*, Transmission electron micrograph showing lamellae parallel to $(100)_{Ol}$ and a larger, faceted spinel crystal which is elongated parallel to $(100)_{Ol}$, but which does not have a lamellar habit. The larger crystal facet (larger arrowhead) is a $\{111\}_{Sp}$ face parallel to $(100)_{Ol}$. The other facets are other $\{111\}$ planes (smaller arrowheads). Selected-area electron diffraction (SAED) of the lamellar region (insert, lower right) shows weak streaking perpendicular to $(100)_{Ol}$ with prominent olivine spots and secondary spinel spots that confirm the topotaxy predicted by Poirier¹⁸. SAED of the larger spinel crystal (insert, lower left) shows that the position and relative intensity of the spots are identical to those of the spinel spots in the lamellar region, confirming that the larger crystal has exactly the same orientation as the lamellae and indicating that it grew from a lamellar nucleus. Scale bars: $20 \mu\text{m}$ in panel *a*; $0.1 \mu\text{m}$ in *b*.

crystals exhibited the topotaxy predicted by Poirier's model, but other crystals displayed the octahedral crystal habit and lacked the topotaxy, making interpretation difficult. In the light of our annealing results, we believe that the simplest explanation for their observations is that stress concentrations produced during pressurization in their apparatus led to production of martensitic nuclei in the earliest stages of their experiments and that coarsening of those nuclei by normal phase-boundary migration during the experiment produced their large, euhedral, crystals. At the temperatures of their experiments, incoherent nuclei were also produced, leading to the crystals that did not display topotaxy with the olivine.

The present results resolve the discrepancies in the literature concerning the mechanism of the transformation in static or quasi-static experiments. The results also imply that the nucleation mechanism should always be martensitic in shock experiments. Indeed, the extreme stresses in shock deformation might lead to complete transformation by the martensitic mechanism rather than the coarsening by normal phase-boundary migration reported here.

One must use caution, however, when extrapolating these results to natural environments, because none of the studies summarized above includes the complication of the β phase^{1,17} (which does not exist for most of the analogue materials, including Mg_2GeO_4). We believe, however, that under most circumstances extrapolation to natural environments should be straightforward. At high temperature and low stress, α transforms to γ by incoherent nucleation and growth in all systems studied, and $\gamma \rightarrow \alpha$ in Mg_2GeO_4 (ref. 21) and $\alpha \rightarrow \beta$ in Co_2SiO_4 (ref. 25) also follow this mechanism. Therefore, in normal mantle environments, where the temperature is high and stresses are very low, the forward or reverse transformation to either β or γ should be accomplished by the reconstructive mechanism. On the other hand, in shocked meteorites the common circumstance should be sudden transport into the γ stability field at low temperature and high stress (that is, by martensitic nucleation). Immediately thereafter, the stress and pressure will fall and the temperature will rise, in some cases leading to the β -phase before the reaction is quenched²⁶⁻²⁸. Downgoing lithospheric slabs present an environment in which both temperatures and stresses are intermediate between these other two environments. Under such conditions, the data are not yet sufficiently comprehensive to rule out either mechanism. We are continuing our experiments at intermediate temperatures and stresses to pursue this question.

It follows from these results that the level of stress could be a factor in experiments aimed at determination of the mechanism or kinetics of other transformations occurring in the deep Earth. In particular, if a martensitic mechanism is possible, then it is to be expected in shock experiments and also is likely in the laser-heated diamond cell. If such mechanisms are observed, their geophysical significance should be interpreted with caution. □

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1. Bina, C. R. & Wood, B. J. *Nature* **324**, 449-451 (1986).
2. Sung, C. & Burns, R. G. *Tectonophysics* **31**, 1-32 (1976).
3. Sung, C. & Burns, R. G. *Earth planet. Sci. Lett.* **32**, 165-170 (1976).
4. Vaughan, P. J., Green, H. W. & Coe, R. S. *Nature* **298**, 357-358 (1982).
5. Boland, J. N. & Liebermann, R. C. *Geophys. Res. Lett.* **10**, 87-90 (1983).
6. Rubie, D. C., Tsuchida, Y., Utsumi, W., Kikigawa, T., Shimomura, O. & Yagi, T. *28th High Pressure Conference of Japan* 132-133 (1987).
7. Yagi, T., Akaogi, M., Shimamura, O., Suzuki, T. & Akimoto, S. *J. geophys. Res.* **92**, 6207-6213 (1987).
8. Remsberg, A. R., Boland, J. N., Gasparik, T. & Liebermann, R. C. *Phys. chem. Miner.* **15**, 498-506 (1988).
9. Vaughan, P. J., Green, H. W. & Coe, R. S. *Tectonophysics* **108**, 299-322 (1984).
10. Rubie, D. C. & Champness, P. E. *Bull. Miner.* **110**, 471-480 (1987).
11. Lacam, A., Madon, M. & Poirier, J.-P. *Nature* **228**, 155-157 (1980).
12. Boland, J. N. & Liu, L. *Nature* **303**, 233-235 (1983).
13. Hamaya, N. & Akimoto, S. in *High Pressure Research in Geophysics* (eds Akimoto, S. & Manghnani, M. E.) 373-389 (Center Academic, Tokyo, 1982).
14. Hamaya, N. & Akimoto, S. *Phys. Earth Planet. Inter.* **29**, 6-11 (1982).
15. Furnish, M. D. & Bassett, W. A. *J. geophys. Res.* **88**, 333-342 (1983).
16. Will, G. & Lauterjung, J. in *High Pressure Research in Mineral Physics* (eds Manghnani, M. H. & Syono, Y.) *Geophys. Monogr.* **39**, 177-186 (American Geophysical Union, Washington, DC, 1987).

17. Green, H. W., *Geophys. Res. Lett.* **11**, 817-820 (1984).
18. Poirier, J.-P. in *Anelastic Properties and Related Process in the Earth's Mantle Geodyn. Ser.* **4**, 113-117 (American Geophysical Union, Washington, DC, 1981).
19. Burnley, P. C. & Green, H. W. *Eos* **69**, 1416-1417 (1988).
20. Green, H. W. *Geophys. Monogr.* **36**, 201-211 (1986).
21. Burnley, P. C. & Green, H. W. *Eos* **68**, 1471 (1987).
22. Green, H. W. & Borch, R. S. *Eur. J. Miner.* (in the press).
23. Webb, S. L., Jackson, I. & Takel, H. *Phys. Chem. Miner.* **11**, 167-171 (1984).
24. Hornstra, J. *J. phys. Chem. Solids* **15**, 311-323 (1960).
25. Remsberg, A. R., Boland, J. N., Gasparik, T. & Liebermann, R. C. *Eos* **68**, 1539 (1987).
26. Putnis, A. & Price, G. D. *Nature* **280**, 217-218 (1979).
27. Price, G. C., Putnis, A. & Agrell, S. O. *Contrib. Miner. Petrol.* **71**, 211-218 (1979).
28. Price, G. D., Putnis, A. & Smith, D. G. W. *Nature* **296**, 729-730 (1982).

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ESR dates for the hominid burial site of Es Skhul in Israel

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THE Middle East has been critical to our understanding of recent human evolution ever since the recovery of Neanderthal and early anatomically modern fossils from the caves of Tabun and Skhul (Mount Carmel) over 50 years ago¹⁻³. It was generally believed, on archaeological and morphological grounds, that middle eastern Neanderthals (such as those from Tabun, Amud and Kebara) probably dated from more than 50,000 years ago, whereas the earliest anatomically modern specimens (from Skhul and Qafzeh) probably dated from about 40,000 years³. Recent thermoluminescence and electron spin resonance (ESR) determinations, however, have supported biostratigraphy in dating the Qafzeh deposits to an earlier part of the late Pleistocene, probably more than 90,000 years ago⁴⁻⁶. These dates have been questioned on unspecified technical grounds^{7,8}, and it has also been argued that they create explanatory problems by separating the morphologically similar Qafzeh and Skhul samples by some 50,000 years, thus implying a long-term coexistence of early modern humans and Neanderthals in the area^{3,7,8}. Here we report the first radiometric dating analysis for Skhul, using ESR on bovine teeth from the hominid burial levels. Early uptake and linear uptake ages average 81 ± 15 and 101 ± 12 kyr respectively. These analyses suggest that the Skhul and Qafzeh samples are of a similar age and therefore it is possible that the presence of early modern humans in the area was episodic, rather than long term during the early late Pleistocene.

The Israeli site of Es Skhul is located in the canyon of Nahal Mearot (Wadi el-Mughareh), near the site of Tabun which has yielded Neanderthal hominid remains. The site of Skhul originally consisted of a 2.5-m thick accumulation of densely cemented, reddish-brown breccia deposited on a triangular rock-cut platform about 11 m above the present level of the wadi floor. McCown⁹, who excavated the site, identified three successively older units. Layer A (<60 cm thick) contained a mixed assemblage of Middle and Upper Palaeolithic artefacts as well as some potsherds. Layer B (a breccia 2 m thick) contained the cranial and post-cranial remains of at least 10 hominids, the majority of which seem to have been intentionally buried, and over 9,800 lithic artefacts representing a Levallois-Mousterian (Middle Palaeolithic) industry. Layer C (a breccia <30-cm thick) contained a sparse industry similar to that in layer B, but no faunal material.

The hominids represent an archaic type of modern *Homo sapiens* and studies of their skeletal morphology demonstrate

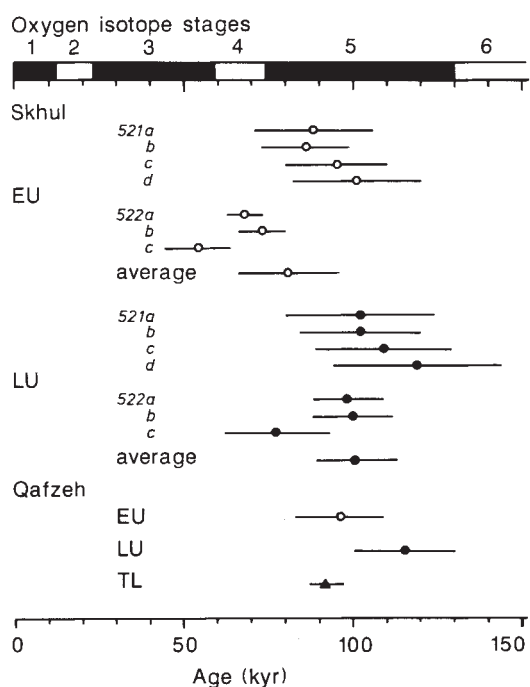


FIG. 1 Horizontal bars represent the ESR ages of two teeth from Skhul. Open circles, ESR ages according to early U-uptake; closed circles, ESR ages according to linear, continuous U-accumulation. The ESR and TL dates on Qafzeh are averages as given by Schwarcz *et al.*⁶ and Valladas *et al.*⁴. The boundaries of the oxygen isotope stages were taken from Martinson *et al.*¹⁵.

clear affinities to those from Qafzeh cave, Israel^{3,10,11}. To establish an absolute age for Skhul we made ESR measurements on two well-preserved bovid teeth from the British Museum (Natural History) collection. Although the exact positions from which the teeth were recovered is not recorded, it is known that they came from the relatively uniform layer B, which contained the hominids.

The procedures of ESR dating have been described by Grün

et al.^{12,13}. The additive dose method was used to determine the acquired dose (AD). The U concentrations of enamel and dentine were determined by neutron activation analyses (Table 1). The external cosmic dose rate was calculated on the assumption that a maximum of 2 m of sediment covered the site. The external β -doses were estimated from analyses of the matrix adjacent to the teeth. Unfortunately, the enclosing matrix had been almost entirely removed by the excavators, so that it was not possible to measure the environmental γ -dose rate at the original site. We collected 16 samples of breccia matrix from the 'Mousterian Layers' of the original 1932 excavation as stored in the British Museum. These samples, plus the sediment samples adjacent to the teeth, were analysed for U (1.86 ± 0.62 p.p.m.), Th (2.18 ± 1.31 p.p.m.), and K ($0.451 \pm 0.225\%$). These values correspond to a total external dose rate of $507 \pm 123 \mu\text{Gy a}^{-1}$, when including a cosmic dose rate of $120 \pm 20 \mu\text{Gy a}^{-1}$ and a water content of $10 \pm 10\%$.

The present-day U content of the enamel (0.3–3.2 p.p.m.) and dentine (6–12 p.p.m.) must have been acquired post-mortem: in calculating the ESR age it can be assumed that U was taken up either soon after burial (early uptake, EU), or gradually (linear uptake, LU; see Fig. 1)^{12,13}. For a given site, LU ages are generally closer to independently determined ages¹⁴ than the minimum possible age defined by EU. The LU and EU ages of the present samples from Skhul average $101,000 \pm 12,000$ years (101 ± 12 kyr) and 81 ± 15 kyr, respectively. The former of these lies between the ESR (LU) and thermoluminescence (TL) dates obtained for Qafzeh (115 ± 15 kyr⁶ and 92 ± 5 kyr⁴, respectively) and is indicative of an early late-Pleistocene age for both sites, corresponding to oxygen isotope stage 5 of the deep-sea record¹⁵.

Schwarcz *et al.*¹⁶ obtained U-series dates on stalagmitic calcite layers adhering to the bedrock or apparently interlaminated with the breccia. The youngest of a series of calcite layers was dated to 79 ± 4 kyr. Although this agrees with the EU age, calcite may have continued to be deposited in this site after the burial was emplaced.

Previous indirect estimates of the age of this site have been based on comparisons of the lithic industry, fauna and hominid remains with those from other nearby sites, especially Tabun. Higgs¹⁷ surmised, on the basis of a faunal analysis, that layer

TABLE 1 ESR results and analytical data for teeth from Skhul

Sample	U(EN) (p.p.m.)	U(DE) (p.p.m.)	U(SED) (p.p.m.)	Th(SED) (p.p.m.)	K(SED) (%)	Thickness* (μm)	Removed* (μm)
521a	0.3	6.0	2.3	1.3	0.27	1200	50
521b	0.3	7.3	2.3	1.3	0.27	1200	50
521c	0.2	7.1	2.2	1.3	0.27	1700	50
521d	0.3	7.5	2.2	1.3	0.27	1500	50
522a	3.2	11.3	3.8	4.2	0.79	1200	20
522b	2.0	10.7	3.8	4.2	0.79	1200	20
522c	3.0	11.5	3.8	4.2	0.79	1200	20

Sample	AD† (Gy)	γ -(SED)‡ ($\mu\text{Gy a}^{-1}$)	β -(SED)§ ($\mu\text{Gy a}^{-1}$)	β -(DE) ($\mu\text{Gy a}^{-1}$)	Early U-uptake		Age (kyr)	β -(DE) ($\mu\text{Gy a}^{-1}$)	Linear U-uptake		Age (kyr)
					Int. ($\mu\text{Gy a}^{-1}$)	Total ($\mu\text{Gy a}^{-1}$)			Int. ($\mu\text{Gy a}^{-1}$)	Total ($\mu\text{Gy a}^{-1}$)	
521a	71.8 ± 9.7	507 ± 123	96.4 ± 11.8	121.3	90.5	815.2 ± 124	88.1 ± 17.9	57.0	40.5	700.9 ± 124	102.0 ± 22.7
521b	72.3 ± 2.6	507 ± 123	96.4 ± 11.8	146.9	89.8	840.1 ± 124	86.1 ± 13.1	68.9	40.0	712.3 ± 124	102.0 ± 18.1
521c	71.9 ± 1.9	507 ± 123	71.7 ± 8.7	115.2	64.0	757.9 ± 123	94.9 ± 15.6	53.9	28.4	661.0 ± 123	109.0 ± 20.5
521d	82.7 ± 9.4	507 ± 123	79.3 ± 9.7	135.2	97.5	807.6 ± 123	101.0 ± 19.0	63.4	43.5	693.2 ± 123	119.0 ± 25.1
522a	123.0 ± 4.7	507 ± 123	213.7 ± 26.1	217.9	869.2	$1,807.6 \pm 126$	68.0 ± 5.4	107.3	422.7	$1,250.7 \pm 126$	98.3 ± 10.6
522b	108.7 ± 4.8	507 ± 123	213.7 ± 26.1	209.6	559.0	$1,489.3 \pm 127$	73.0 ± 7.0	102.0	265.9	$1,088.6 \pm 126$	99.9 ± 12.4
522c	91.5 ± 15.9	507 ± 123	213.7 ± 26.9	211.3	745.3	$1,677.3 \pm 126$	54.6 ± 10.3	104.0	361.2	$1,185.9 \pm 126$	77.2 ± 15.7

EN=enamel; DE=dentine; SED=sediment; β -()= β -dose rate; γ -()= γ -dose rate; int.=internal dose rate.

* The values for thickness of enamel and the layers removed from both sides (to eliminate the volume irradiated by external α -rays) were used to calculate the β -attenuation factors²².

† ADs and uncertainties were calculated with the computer program FITT²³.

‡ The uncertainty of the external γ -dose rate consists of the variation of radioactive elements in the sediment samples analysed by NAA and a systematic error for variation of water content⁴.

§ The uncertainty of the external β -dose is caused by a systematic error of water content⁴.

|| The analytical error in the determination of U, Th, and K is negligible for the calculation of the respective dose rates.

B at Skhul might be 10 kyr younger than layer C at Tabun, for which a ^{14}C date of 51 kyr has been obtained¹⁸. Jelinek¹⁸ compared the Tabun and Skhul artefacts and concluded that those from Skhul were less than 50 kyr old. Such estimates, as well as morphological dating, have been used to place the Skhul hominids at about 40 kyr³.

By contrast, our results show that the ESR dates for teeth associated with the Skhul hominids are indistinguishable from, or only slightly younger than those previously obtained⁶ for anatomically similar hominids from Qafzeh. As at Qafzeh, the hominids are associated with a Middle Palaeolithic industry similar to that used at a later time by morphologically distinct Neanderthals elsewhere in this region^{19,20}. It is unclear whether this precludes important behavioural differences between the early modern humans and Neanderthals in respects other than lithic industry. The Skhul and Qafzeh dates indicate the existence of anatomically modern hominids in south-west Asia long before their appearance in Europe, and at approximately the same time as their estimated earliest appearance in Africa^{5,21}. The extent of any coexistence between early modern humans and Neanderthals in south-west Asia remains unknown, and documentation will depend on further dating work, particularly for Neanderthal sites such as Tabun, Amud and Shanidar. Clarification of the chronological and phylogenetic relationships between the Skhul-Qafzeh hominids and the more fragmentary and archaic humans known from sites such as Gesher Benot Ya'acov, Zuttiyeh and Tabun E is also required³.

An early late-Pleistocene age for both the Qafzeh and Skhul samples suggests at least two possibilities for this period of human evolution in south-west Asia. First, the presence of the early moderns might reflect a brief episodic occupation in the area (perhaps from north Africa), preceded by archaic humans associated with the Acheulo-Yabrudian, and followed by archaic humans (Neanderthals), also associated with the Middle Palaeolithic. Alternatively, archaic and early modern humans may have alternated or overlapped in occupation of the area through the Middle Palaeolithic. It remains unclear, however, whether the early late-Pleistocene presence of modern humans in south-west Asia presaged an early spread to east Asia (and much later, into Europe), or whether the further radiation of modern people into both Eurasia and the Far East was later, and unconnected with the evidence from Skhul and Qafzeh⁵. □

A Middle Palaeolithic human hyoid bone

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THE origin of human language, and in particular the question of whether or not Neanderthal man was capable of language/speech, is of major interest to anthropologists but remains an area of great controversy^{1,2}. Despite palaeoneurological evidence to the contrary^{3,4}, many researchers hold to the view that Neanderthals were incapable of language/speech, basing their arguments largely on studies of laryngeal/basicranial morphology^{1,5,6}. Studies, however, have been hampered by the absence of unambiguous fossil evidence. We now report the discovery of a well-preserved human hyoid bone from Middle Palaeolithic layers of Kebara Cave, Mount Carmel, Israel, dating from about 60,000 years BP. The bone is almost identical in size and shape to the hyoid of present-day populations, suggesting that there has been little or no change in the visceral skeleton (including the hyoid, middle ear ossicles, and inferentially the larynx) during the past 60,000 years of human evolution. We conclude that the morphological basis for human speech capability appears to have been fully developed during the Middle Palaeolithic.

The Kebara hyoid is part of a nearly complete Middle Palaeolithic skeleton (Kebara 2) unearthed during the 1983 joint French-Israel excavations^{7,8}. Figure 1 gives an anterior view of the bone. It is nearly complete—the body and the two greater horns are preserved. The latter are not fused to the body of the bone and the lesser horns are missing. Both of these features are also common in collections of modern human hyoids, because synostosis between the various elements of the hyoid is not always achieved, and in fact the small horns may remain cartilaginous during an individual's lifetime.

The ventral surface of the body presents two deep superior fossae separated by a median crest for the attachment of the geniohyoid muscle, and two less marked latero-inferior fossae for the omohyoid muscle. In a posterior (dorsal) view, the facets for the greater horns are very evident and the surface of the hyoid body is concave and rugged.



FIG. 1 The hyoid bone from Kebara 2 seen in anterior view.

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1. *The Stone Age of Mount Carmel Vol. I* (eds Garrod, D. A. E. & Bate, D. M. A.) (Clarendon, Oxford, 1937).
2. *The Stone Age of Mount Carmel Vol. II* (eds McCown, T. D. & Keith, A.) (Clarendon, Oxford, 1939).
3. Trinkaus, E. in *The Origin of Modern Humans* (eds Smith, F. H. & Spencer, F.) 251–293 (Liss, New York, 1984).
4. Valladas, H. et al. *Nature* **331**, 614–616 (1988).
5. Stringer, C. *Nature* **331**, 565–566 (1988).
6. Schwarcz, H. P., Grün, R., Vandermeersch, B., Bar-Yosef, O. & Valladas, H. *J. hum. Evol.* **17**, 733–737 (1988).
7. Bower, B. *Science News* **133**, 138 (1988).
8. Wolpoff, M. H. *Am. J. phys. Anthropol.* **78**, 326 (1988).
9. McCown, T. D. in *The Stone Age of Mount Carmel Vol. I* (eds Garrod, D. A. E. & Bate, D. M. A.) 91–107 (Clarendon, Oxford, 1937).
10. Vandermeersch, B. *Les Hommes Fossiles de Qafzeh (Israël)* (CNRS, Paris, 1981).
11. Stringer, C. B. & Trinkaus, E. in *Aspects of Human Evolution* (ed Stringer, C. B.) 129–165 (Taylor & Francis, London, 1981).
12. Grün, R. *Die ESR-Altersbestimmungsmethode* (Springer, Heidelberg-Berlin, 1989).
13. Grün, R., Schwarcz, H. P. & Zymela, S. *Can. J. Earth Sci.* **24**, 1022–1037 (1987).
14. Schwarcz, H. P., Grün, R., Latham, A. G., Mania, D. & Brunnacker, K. *Archaeometry* **30**, 5–17 (1988).
15. Martinson, D. G. et al. *Quat. Res.* **27**, 1–29 (1987).
16. Schwarcz, H. P., Goldberg, P. & Blackwell, B. *Israel J. Earth Sci.* **29**, 157–165 (1980).
17. Higgs, E. S. *Proc. prehist. Soc.* **27**, 144–154 (1961).
18. Jelinek, A. *Science* **216**, 1369–1375 (1982).
19. Valladas, H. et al. *Nature* **330**, 159–160 (1987).
20. Schwarcz, H. P., Buhay, W. M., Grün, R., Valladas, H. & Tchernov, E. *J. arch. Sci.* (in the press).
21. Deacon, H. J. & Geleijnse, V. B. *South African arch. Bull.* **43**, 5–14 (1988).
22. Grün, R. *Ancient TL* **4**, 1–8 (1986).
23. Grün, R. & Macdonald, P. D. M. *Appl. Radiat. Isotopes* (in the press).

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The right greater horn is complete and presents at its proximal end a large articular facet for the body of the hyoid, and at its distal end a small faint attachment surface for the thyrohyoid ligament. The left greater horn is missing its distal end. The principal measurements of the various elements of the hyoid bone are given in Table 1.

The metric traits of the Kebara bone are compared with the hyoid bones of a large sample of anatomically modern humans. The only consistent differences between the fossil bone and those of recent humans are in the maximum medial height of the body and depth of the posterior surface. Yet the former measurement is within the modern human range of variation; only the latter measurement exceeds the means and maximum range found in modern samples. The transverse diameter of the complete hyoid also appears to be large (Table 1), but the accurate position of the greater horns to the body is difficult to assess because of the missing articular cartilages.

Some other bones of the Kebara 2 skeleton present strongly marked metric and morphological differences with like bones of modern humans. For example, the mandible displays four dimensions (bicondylar breadth, bigonial breadth, bimental foramen breadth, and maximum body length) which are beyond

three standard deviations from the means of a sample of modern Bedouin mandibles. Indeed, the unusually large size of the Kebara 2 mandible implies a far greater robusticity of the muscles that relate this bone to the hyoid (for example, mylohyoid, geniohyoid, genioglossus, digastric) than in the Bedouin sample. The differences in the areas of attachment of muscles in Kebara 2 and modern humans are observed only in the mandible and are not reflected in the hyoid bone where insertions remain quite small, as in present-day populations. The hyoid of Kebara 2 thus presents a notable case of morphological stability through time, in sharp contrast to the mandible.

The relatively few morphological and metric changes that have occurred in the hyoid during the last 60,000 (and perhaps 90,000) years have been observed in other visceral bones. There is a marked similarity among the middle ear ossicles of Middle Palaeolithic Neanderthals, early anatomically modern humans, and present-day populations⁹⁻¹¹. Together, the evidence from the hyoid and middle ear ossicles suggests that the osseous elements derived from the embryonic branchial arches show less rapid evolutionary changes than bones derived from dermal or enchondral tissues. A related inference would be that the associated larynx beneath the hyoid has scarcely changed in

TABLE 1 Measures (mm) of the Kebara hyoid bone compared with those of other groups

Group and period	Maximum transverse diameter*	Maximum medial height†	Anterior-posterior thickness‡	Depth of posterior surface§	Total sagittal diameter	Total transverse diameter¶
Kebara	24.6	13.4	5.8	3.8	35.5	45.0
Hayonim (Natufian)						
N	4	5	5	5	—	—
Mean + s.d.	22.00 0.73	11.04 1.17	5.78 0.69	2.28 0.29	—	—
Maximum	22.6	12.4	6.3	2.6	—	—
Minimum	21.0	10.0	4.6	1.8	—	—
La Boucle à Coronne (Neolithic)						
N	11	18	18	18	3	4
Mean + s.d.	20.74 1.56	10.37 0.92	4.85 0.72	2.03 0.68	33.33 2.90	38.78 1.31
Maximum	22.3	11.8	6.4	3.1	36.5	40.2
Minimum	18.3	8.5	3.7	0.8	30.8	37.2
La Cauno à Moux (Chalcolithic)						
N	5	12	10	11	—	—
Mean + s.d.	20.58 3.41	10.14 1.37	4.59 0.61	2.05 0.62	—	—
Maximum	23.6	11.7	5.3	3.0	—	—
Minimum	15.7	7.5	3.2	1.2	—	—
Ein Gedi (Roman period)						
N	15	15	15	15	—	—
Mean + s.d.	21.88 3.75	11.30 1.77	5.59 0.93	2.22 0.60	—	—
Maximum	27.2	14.2	7.8	3.4	—	—
Minimum	14.4	8.4	4.2	1.4	—	—
Bedouins (Recent period)						
N	13	17	17	17	8	8
Mean + s.d.	21.35 2.07	10.66 0.77	5.15 1.25	1.77 0.48	36.53 3.02	39.06 3.27
Maximum	25.4	12.0	9.0	2.5	39.8	43.5
Minimum	18.3	9.5	3.8	0.6	30.0	34.4
Total modern human sample						
N	48	67	65	66	11	12
Mean + s.d.	21.35 2.66	10.66 1.27	5.13 0.97	2.03 0.59	35.65 3.20	38.97 2.70

Samples from La Boucle à Coronne and La Cauno à Moux are from H. Duda excavations, housed in the Laboratoire d'Anthropologie, Université de Bordeaux 1, Talence, France. All other samples are from the Department of Anatomy and Anthropology, Tel Aviv University, Israel.

* Maximum transverse diameter of body.

† Maximum medial height of body.

‡ Antero-posterior thickness of body in sagittal plane: one caliper branch tangent to the upper and to the lower posterior margins of the body, the other branch tangent to the most protruding antero-medial point.

§ Depth of the posterior surface of the body: measured from the upper and the lower posterior margins in the sagittal plane.

|| Total maximum length of the bone in the sagittal plane: in projection from distal end of the greater horns to most medio-anterior point of the body, with the greater horns being attached to the body in their anatomical position.

¶ Total maximum breadth of the bone in transverse plane: taken at the most external distal ends of the greater horns, with the greater horns attached to the body in their anatomical position.

position, form, relationships or size during the past 60,000 years of human evolution. If indeed this inference is warranted, the morphological basis for human speech capability appears to have been fully developed during the Middle Palaeolithic, contrary to the views of some researchers^{1,5,6}.

The discovery of a fossil hyoid with strong similarities to hyoids of modern populations permits a new perspective on the Middle Palaeolithic human ability to communicate in an articulate language, with far-reaching implications regarding behaviour. The assumed speech limitations of the Neanderthals, that have hitherto been based primarily on studies of basicranial morphology, would seem to require revision. □

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1. Lieberman, P. *The Biology and Evolution of Language* (Harvard Univ. Press, Cambridge, 1984).
2. Wind, J. *Actes du Colloque International de Liege. Etudes et Recherches Archeologiques de l'Université de Liege*, Vol. 32, 117–123 (1988).
3. Holloway, R. L. *Hum. Neurobiol.* **2**, 105–114 (1983).
4. Tobias, P. V. *J. hum. Evol.* **16**, 741–761 (1987).
5. Crelin, E. S. *The Human Vocal Tract* (Vantage, New York, 1987).
6. Laitman, J. T. in *Hominid Evolution, Past Present and Future* (ed. Tobias, P. V.) 181–186 (Liss, New York, 1985).
7. Arensburg, B. et al. *C. r. Acad. Sci., Paris* **300/11**, 227–230 (1985).
8. Valladas, H. et al. *Nature* **330**, 159–160 (1987).
9. Arensburg, B. & Nathan, H. *L'Anthropologie* **76**, 301–307 (1972).
10. Heim, J. L. *Les Enfants Neandertaliens de la Ferrassie* (Masson, Paris, 1982).
11. Arensburg, B. & Tillier, A. M. *Bull. Mem. Soc. Anthropol., Paris* **10**, 61–69 (1983).

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A cost of mating in female fruitflies

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COSTS of reproduction occur when an increase in reproductive rate reduces future reproduction by increasing mortality or reducing fertility. Such costs have been demonstrated in plants and animals in laboratory and field studies^{1–5}. Their importance lies in their possible role in the evolution of life histories and of senescence^{6–15}. An understanding of their mechanisms will also reveal the nature of the physiological constraints on longevity and fertility. Most accounts assume that these occur as a result of competition for nutrient allocation between growth, storage, somatic maintenance and reproduction^{16–21}. An increase in reproductive rate would then result in a denial of nutrients to other processes, resulting in a drop in life expectancy or future fertility. Some support for this point of view comes from the finding that lifespan is lengthened in female *Drosophila melanogaster* that have inactive or absent ovaries^{22–23} or that are experimentally induced to produce fewer eggs²⁴. Increased exposure to males, however, also results in a drop in lifespan^{24–26}. We show here that mating with males greatly reduces lifespan in female fruitflies whose rates of egg-production and egg-fertility do not differ, suggesting both that simple nutrient allocation to reproduction is not its only physiological cost, and that males can cause females to remate at a frequency that results in reduced female lifetime reproductive success.

Wild-type Dahomey virgin females were collected at eclosion from uncrowded cultures and allocated randomly to two experimental groups, each of 44 flies. The aim was to vary the opportunity for mating between the groups, but to equalize them for both production of fertile eggs and all aspects of pre-mating exposure to males. This was done by keeping each female in the 'high-mating' group permanently with two wild-type males,

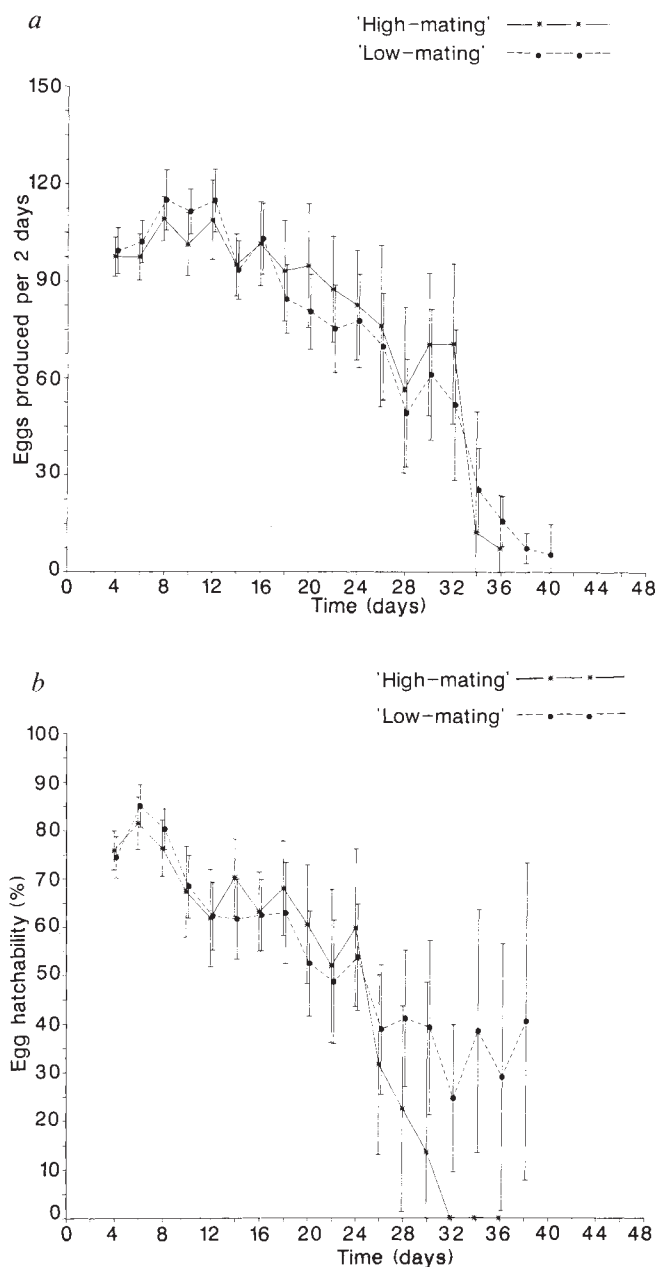


FIG. 1 *a*, Means (and 95% confidence limits) for the number of eggs produced in each 2-day interval. *b*, Means (and 95% confidence limits) of egg-hatchability, measured for each female as the proportion of eggs laid producing adult progeny for each 2-day time interval.

initially 4-days old and renewed every 10 days. The females in the 'low-mating' group were kept with two wild-type males for 1 day out of 3, and on the remaining 2 days with two males that had had their external genitalia ablated by microcautery. These males courted normally (Table 1), and survived as well in the experiment as intact males, but they could not mate with the females; this was checked before the males were introduced to the experiment, by keeping them individually with two, initially 3-day-old, virgin females for 3 days, and checking for production of larvae. Exposure to microcauterized males resulted in levels of female activity that were not significantly different from those seen in females exposed to intact males (Table 2). All females were kept in 75×24 mm shell vials over 7 ml of freshly yeasted Lewis medium at 25 °C on a 12:12 light-dark cycle, and were transferred to fresh vials every 2 days. All flies were handled under CO₂ anaesthesia. All eggs produced were

counted every 2 days, and the vials were retained for a count of the adults emerging, to give a measure of egg-hatchability. In a separate but identical experiment, the amount of mating by the two groups was measured on the one day in three when the 'low-mating' females were exposed to intact males.

The two groups of females showed no significant differences in the numbers of eggs produced (Fig. 1a), nor in the hatchability of eggs (Fig. 1b). The 'high-mating' group remated more frequently than the 'low-mating' group; on the day when both groups had intact males, the proportion of females remating did not differ significantly (Table 1). If these data are extrapolated to include the 2 out of 3 days when the 'low-mating' females had microcauterized males, females in the 'high-mating' group remated significantly more frequently. The 'high-mating' group had significantly lower lifespans than the 'low-mating' group (Fig. 2).

These results show that mating *per se* reduces female lifespan. The mechanism of the effect is at present unknown; it could be a consequence of mechanical injury, transfer of parasites, or an effect of sperm or accessory fluid components on the females. Transfer of parasites is an unlikely explanation, because the intact males used in the experiment were all virgins, and any non-venereal source of infection for males should also have been encountered by females. Use of mutant males may enable the mechanisms to be deduced. Our results suggest that diversion of nutrients into reproduction is unlikely to account for the reduction in lifespan, because the increase in mating was not accompanied by any increase in reproductive output. Females that lay more eggs remate more frequently²⁷, so any increase in egg-production would be likely also to incur an additional mating cost.

The females in the 'high-mating' group suffered a drop in lifetime reproductive success as a result of their higher mating rate because their lifespan was reduced, but there was no increase in age-specific progeny production. They would therefore have had increased fitness if they had mated less frequently. Males always gain from a mating, and the finding implies that males can somehow persuade females to mate when it is not in their long term reproductive interests to do so. One possibility is that this is an artefact of the levels of exposure to males used in the experiment, so that the phenomenon would not occur in nature. The frequencies of remating that we have observed, however, are lower than those previously seen in natural or

TABLE 1 Numbers of females mating and courted

	Mating	Not mating
'High-mating'	15	264
'Low-mating'	25	983
	Courtship	No courtship
'High-mating'	97	173
'Low-mating'	108	166

Number of females mating in 33 h of direct observation in the 3 h after lights on, spread evenly over 11 days in the first 36 days of the experiment. For both groups the 'mating' total is the number of females mating on the sampling days. For the 'high-mating' group, the 'not mating' total is the number of females present on the sampling days that did not mate. For the 'low-mating' group, the 'not-mating' total is the number of females present on the sampling days that did not remate, plus the number of females present on the day before and the day after the sampling day. χ^2 1 d.f. = 6.1, $P < 0.02$. Lower part of table shows number of females courted in spot samples within 3 h of lights on and spread throughout the days of the experiment, on days when the 'low-mating' females were exposed to microcauterized males. Male courtship was defined as wing vibration or attempted copulation. χ^2 1 d.f. = 0.71, $p > 0.4$.

TABLE 2 Levels of female activity

	Courtied		Not courtied	
	Moving	Still	Moving	Still
'High mating'	24	73	28	145
'Low mating'	27	81	30	136

Spot samples of female movement (walking or running) while courtship was or was not occurring. Observations were spread evenly through the experiment, on days when the 'low-mating' females were exposed to microcauterized males. For courtship samples, χ^2 1 d.f. = 0.002 NS, for non-courtship samples χ^2 1 d.f. = 0.213 NS.

semi-natural populations²⁸. This seems, therefore, to be a case where a conflict of interest between the sexes has been at least temporarily resolved in favour of males. This result does not support the idea that multiple mating may be of advantage to females²⁹. □

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1. Bell, G. & Koufapanou, V. in *Oxford Surveys in Evolutionary Biology* Vol. 3 (eds Dawkins, R. & Ridley, M.) 83-131 (Oxford University Press, 1985).
2. Reznick, D. *Oikos* **44**, 257-267 (1985).
3. Partridge, L. & Harvey, P. H. *Science* **241**, 1449-1454 (1988).
4. Gustafsson, L. & Sutherland, W. J. *Nature* **335**, 813-815 (1988).
5. Partridge, L. in *Lifetime Reproduction in Birds* (ed. Newton, I.) (Academic, London, in the press).
6. Williams, G. C. *Evolution* **11**, 398-411 (1957).
7. Williams, G. C. *Am. Nat.* **100**, 687-690 (1966).
8. Hamilton, W. D. *J. theor. Biol.* **12**, 12-45 (1966).
9. Gadgil, M. & Bossert, W. *Am. Nat.* **104**, 1-24 (1970).
10. Charnov, E. L. & Krebs, J. R. *Ibis* **116**, 217-219 (1974).
11. Planka, E. R. & Parker, W. S. *Am. Nat.* **109**, 453-464 (1975).
12. Charlesworth, B. *Evolution in Age-Structured Populations* (Cambridge University Press, 1980).
13. Partridge, L. *Funct. Ecol.* **1**, 317-320 (1987).
14. Rose, M. R. *Evolution* **38**, 1004-1010 (1984).
15. Luckinbill, L. S. et al. *Evolution* **38**, 996-1003 (1984).
16. Fisher, R. A. *The Genetical Theory of Natural Selection* (Dover, New York, 1958).
17. Kirkwood, T. B. L. *Nature* **270**, 301-304 (1977).
18. Calow, P. *Biol. Rev.* **54**, 23-40 (1979).
19. Kirkwood, T. B. L. in *Physiological Ecology* (eds Townsend, C. R. & Calow, P.) 165-189 (Blackwell Scientific Publications, Oxford, 1981).
20. Kirkwood, T. B. L. & Holliday, R. *Proc. R. Soc. B* **205**, 531-546 (1979).
21. Bryant, D. M. *Funct. Ecol.* **2**, 23-34 (1988).
22. Maynard Smith, J. *J. exp. Biol.* **35**, 832-842 (1958).
23. Lamb, M. J. *J. Insect Physiol.* **10**, 487-497 (1964).
24. Partridge, L., Green, A. & Fowler, K. *J. Insect Physiol.* **33**, 745-749 (1987).
25. Partridge, L. et al. *J. Insect Physiol.* **32**, 925-929 (1986).
26. Luckinbill, L. S. et al. *Evol. Ecol.* **2**, 85-94 (1988).
27. Trevitt, S., Fowler, K. & Partridge, L. *J. Insect Physiol.* **34**, 821-828 (1988).
28. Partridge, L., Hoffmann, A. & Jones, J. S. *Anim. Behav.* **35**, 468-476 (1987).
29. Walker, W. F. *Am. Nat.* **115**, 780-799 (1980).

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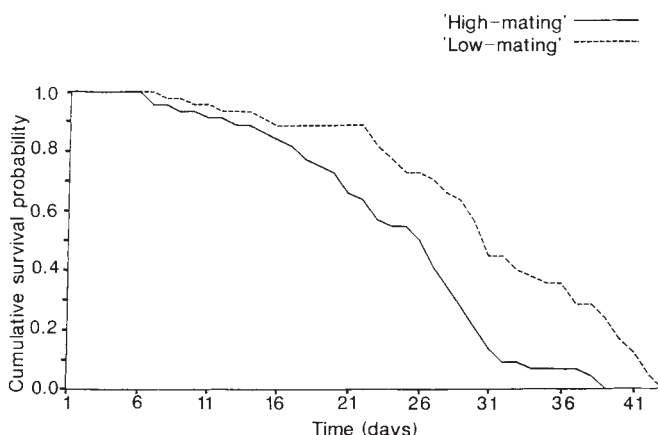


FIG. 2 Survival curves for 'high-mating' and 'low-mating' females. Cumulative survival probability is the proportion of females still alive at the end of each sampling interval. χ^2 1 d.f. = 15.4, $P < 0.001$ Log Rank Test (this test compares the observed numbers of deaths in each group in each sampling interval with the numbers expected if the observed deaths occurred in proportion to the numbers of flies present in each group).

5-HT₃ receptors mediate inhibition of acetylcholine release in cortical tissue

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THE release of cerebral acetylcholine from terminals in the cerebral cortex has been shown to be regulated by 5-hydroxytryptamine (5-HT)¹⁻⁶ but it is not known which subtype of the 5-HT receptor is involved. 5-HT receptor agonists increase acetylcholine levels *in vivo*^{1,2}, indicating a reduced turnover, and reduce release of acetylcholine from striatal slices *in vitro*³⁻⁵. Depleting 5-HT by inhibiting synthesis or by destroying the neurons containing 5-HT potentiates acetylcholine release⁵, and increases acetylcholine turnover in the cerebral cortex and hippocampus⁶. Selective antagonists for the 5-HT₃ receptor subtypes which seem to have effects on mood and activity⁷⁻¹⁰ may exert their effect through the regulation of acetylcholine release in the cortex and limbic system. Radioligand binding studies show a high density of 5-HT₃ receptors in the cholinergic-rich entorhinal cortex^{11,12} and we provide evidence that a reduction in cortical cholinergic function can be effected *in vitro* by 5-HT₃ receptors.

Release of [³H]acetylcholine from rat entorhinal cortex preloaded with [³H]choline was stimulated by potassium. Previous studies have shown that the tritium released is [³H]acetylcholine, and that the release is calcium-dependent¹³. 5-HT (2 µM) or the selective 5-HT₃ receptor agonist, 2-methyl-5-HT (ref. 14; 2 µM) inhibited potassium-stimulated [³H]acetylcholine release by some 50% (*P* < 0.001), but only in the presence of the 5-HT₂/5-HT_{1C} receptor antagonist ritanserin¹⁵ (Table 1). Alone, the 5-HT₃ receptor antagonists GR38032F (ref. 16) and

zacopride¹⁷ (both at 1 nM) failed to alter basal or evoked [³H]acetylcholine release, although they antagonized the reduction in evoked release induced by 2-methyl-5-HT (2 µM) in the presence of ritanserin (1 µM) (Table 1). In the absence of ritanserin, 2-methyl-5-HT in the presence of GR38032F or zacopride caused 107 and 37% increases in potassium-stimulated [³H]acetylcholine release, respectively (Table 1). The ritanserin sensitivity of this excitatory effect suggests mediation

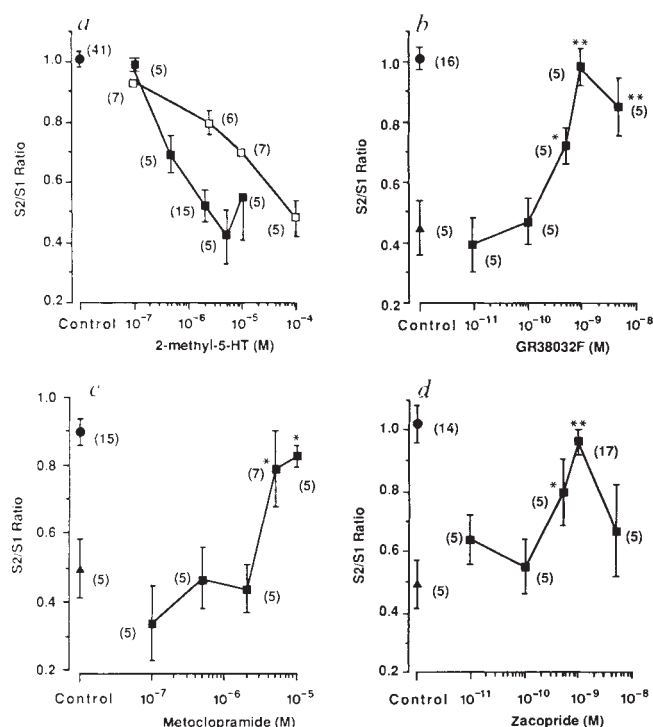


FIG. 1 Inhibition of potassium-stimulated [³H]acetylcholine release by 2-methyl-5-HT (0.1–10.0 µM) and antagonism by metoclopramide (0.1–10.0 µM), GR38032F (0.01–10.0 nM) or zacopride (0.01–10.0 nM). Ritanserin (1 µM) was used in all experiments. The entorhinal cortex from female, Hooded Lister rats (250–300g, Bradford bred) was cross-chopped at 90° (McIlwain tissue chopper) to produce slices 0.35 × 0.35 mm × thickness of cortical ribbon. Tissue was obtained from 20 rats for each experiment and each chamber contained tissue from a single rat. The cut tissue was washed three times in gassed (95% O₂/5% CO₂) Krebs solution (NaCl 118.0, KCl 4.75, KH₂PO₄ 1.2, MgSO₄ · 7H₂O 1.2, CaCl₂ 2.5, NaHCO₃ 25.0, glucose 11.0 mM). The Krebs solution was then replaced by one containing 39.75 mM KCl (tonicity was maintained by reducing the NaCl concentration) and the tubes shaken at 37 °C for 20 min. After being washed the slices were incubated at 37 °C for 40 min in normal Krebs buffer containing [³H]choline, 0.1 µM (15 Ci mmol⁻¹, Amersham). 200 µl washed settled slices were placed into each of the 20 superfusion baths and superfused with Krebs solution (0.5 ml min⁻¹) containing 1.0 µM hemicholinium-3 at 37 °C. After a 30 min washout period, 4-min samples of perfusate were collected. At 12 min (S₁) and 48 min (S₂) the slices were depolarized by changing the superfusion fluid for 4 min to a Krebs solution containing 20 mM KCl. The superfusate samples were collected for a total of 80 min. Tritium content was assayed by liquid scintillation spectroscopy (Tri-Carb 1900CA, efficiency 47%). The evoked release of radioactivity was calculated as the difference between potassium-stimulated and basal release. On completion of the experiment, the tritium remaining in the slices was determined. The disintegration per min, per 4-min collection period, was converted to fractional release by dividing by the total amount of radioactivity present in the tissue at the end of each 4-min collection period¹³. Ordinates, mean S₂/S₁ ratio ± s.e. calculated from *n* determinations. Abscissa, concentrations of agonist or antagonists. *a*, Concentration-response curves to 2-methyl-5-HT in the absence (■) and presence (□) of GR38032F (10 nM). *b*, *c* and *d*, Antagonism of 2-methyl-5-HT (▲, 2 µM) by GR38032F, metoclopramide and zacopride, respectively. Significant differences from 2-methyl-5-HT (2 µM) response are shown as **P* < 0.05, ***P* < 0.01; ●, control response.

TABLE 1 Influence of 5-HT agonists and antagonists on [³H]acetylcholine release

Treatment	Evoked [³ H]acetylcholine release S ₂ /S ₁ ratio (<i>n</i>)	
	Control	Treatment
5-HT	0.90 ± 0.03 (5)	0.77 ± 0.06 (4)
5-HT + Ritanserin	1.05 ± 0.03 (5)	0.51 ± 0.06 (4)*
2-Methyl-5-HT	0.98 ± 0.06 (5)	1.11 ± 0.14 (5)
2-Methyl-5-HT + Ritanserin	0.96 ± 0.06 (10)	0.52 ± 0.05 (15)*
Ritanserin	1.06 ± 0.07 (8)	0.91 ± 0.07 (5)
GR38032F	0.98 ± 0.06 (5)	1.04 ± 0.11 (3)
2-Methyl-5-HT + GR38032F	0.95 ± 0.03 (6)	1.97 ± 0.21 (6)*
2-Methyl-5-HT + GR38032F + Ritanserin	0.99 ± 0.04 (14)	0.98 ± 0.06 (12)†
Zacopride	1.01 ± 0.10 (5)	0.99 ± 0.05 (3)
2-Methyl-5-HT + Zacopride	0.98 ± 0.06 (5)	1.34 ± 0.15 (6)*
2-Methyl-5-HT + Zacopride + Ritanserin	1.01 ± 0.04 (14)	0.98 ± 0.04 (12)†

Influence of 5-HT agonists and antagonists, alone and in combination, on potassium-stimulated (20 mM) release of [³H]acetylcholine from slices of rat entorhinal cortex (see Fig. 1). All test drugs were added 20 min before, during and after the S₂ stimulation, and the S₂/S₁ ratio was subsequently calculated: 5-HT (2 µM), 2-methyl-5-HT (2 µM), ritanserin (1 µM), GR38032F (1 nM), zacopride (1 nM). Mean S₂/S₁ ratios ± s.e. and the numbers of rats used (*n*) for each determination are shown. Significant decreases or increases of S₂/S₁ ratios compared with corresponding controls were determined using Dunnett's *t* test (*P* < 0.01*). † Indicates significant antagonism of these effects by GR38032F, zacopride or ritanserin (*P* < 0.01).

through 5-HT₂ or 5-HT_{1C} sites; although the involvement of 5-HT₂ receptors may be questioned as other functional studies have shown 2-methyl-5-HT to have little effect at the concentrations used in the present studies¹⁴.

The inhibitory effect of 2-methyl-5-HT on potassium-stimulated [³H]acetylcholine release was concentration-dependent in the 0.5–10.0 µM range, which is typical of its potency for 5-HT₃ receptor-mediated responses^{14,16,18,19}. Metoclopramide (5–10 µM), GR38032F (0.5–5.0 nM) and zacopride (0.5–1.0 nM) showed a concentration-dependent antagonism on the inhibitory effect of 2-methyl-5-HT (2 µM) (Fig. 1). The pIC₅₀ value (±s.e.) for zacopride, calculated from the rising portion of the antagonist response curve, was 9.38 ± 0.08 (sample size, *n* = 5) which is similar to reported values (5-HT₃ binding pK_i = 8.70 (ref. 12), functional pA₂ values 8.5–10.1 (ref. 17)). The pIC₅₀ value for metoclopramide (5.80 ± 0.09; *n* = 5), however, was slightly lower than expected (5-HT₃ binding pK_i 6.45–6.49 (refs 11 and 12), functional pA₂ values 6.6–7.2 (refs 19 and 20)), which may reflect an antagonism of dopamine receptors.

In further concentration-response studies, GR38032F (10 nM) caused a parallel rightward shift in the 2-methyl-5-HT inhibitory concentration-response curve without changing the maximum response (dose ratio 15.6, Fig. 1). The competitive affinity (pK_B ± s.e.) for the antagonist GR38032F was 9.04 ± 0.12 (*n* = 5); this value is similar to those reported previously in functional systems (pA₂ 8.13–9.40 (ref. 16)) and binding assays (pK_i 8.32–8.54 (refs 11 and 12)).

The specificity for 5-HT₃ receptors of the 2-methyl-5-HT (2.0 µM) inhibitory effect (in the presence of 1 µM ritanserin) was confirmed by the failure of other antagonists to influence the evoked [³H]acetylcholine release or its inhibition by 2-methyl-5-HT (namely, methysergide, fluphenazine, prazosin, propranolol, sulpiride, idazoxan, naloxone, atropine, mepyramine, ranitidine and chlordiazepoxide, all at 1 µM).

Two concepts emerge from these *in vitro* studies. First, activation of 5-HT₃ receptors can reduce acetylcholine release from the cerebral cortex, and, second, 5-HT₃ receptor antagonists may act by facilitating acetylcholine release. Such a facilitation could be achieved either by directly preventing the inhibitory effect of a 5-HT₃-receptor agonist or by allowing a facilitatory agonist to act at putative 5-HT₂/5-HT_{1C} receptors (ritanserin-sensitive sites). The increases in cortical acetylcholine release we report were effected by 5-HT receptor antagonists at affinities consistent with those determined in other 5-HT₃ receptor models (refs 11, 12, 16, 17, 19 and 20). GR38032F and zacopride, highly selective 5-HT₃ receptor antagonists, are particularly potent, and these novel psychotherapeutic agents may provide a link in the search for relieving the consequences of impaired cortical cholinergic function. □

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1. Euvrard, C., Javoy, F., Herbert, A. & Glowinski, J. *Eur. J. Pharmac.* **41**, 281–289 (1977).
2. Ladinsky, H., Consolo, S., Peri, G., Crunelli, V. & Samanin, R. in *Cholinergic Mechanisms and Psychopharmacology* (Ed. Jenden, D.J.) 615–627 (Plenum, New York, 1978).
3. Gillet, G., Ammor, S. & Fillion, G. *J. Neurochem.* **45**, 1687–1691 (1985).
4. Jackson, D., Bruno, J. P., Stachowiak, M. K. & Zigmond, M. J. *Brain Res.* **457**, 259–266 (1988).
5. Vizi, E. S., Harsin, L. G. & Zsilla, G. *Brain Res.* **212**, 89–99 (1981).
6. Robinson, S. E. *Life Sci.* **32**, 345–353 (1983).
7. Costall, B., Domeney, A. M., Naylor, R. J. & Tyers, M. B. *Br. J. Pharmac.* **92**, 881–894 (1987).
8. Hagan, R. M. *et al. Eur. J. Pharmac.* **138**, 303–305 (1987).
9. Costall, B., Domeney, A. M., Gerrard, P. A., Kelly, M. E. & Naylor, R. J. *J. Pharm. Pharmac.* **40**, 302–305 (1988).
10. Jones, B. J. *et al. Br. J. Pharmac.* **93**, 985–993 (1988).
11. Kilpatrick, G. J., Jones, B. J. & Tyers, M. B. *Nature* **330**, 746–748 (1987).
12. Barnes, N. M., Costall, B. & Naylor, R. J. *J. Pharm. Pharmac.* **40**, 548–551 (1988).
13. Barnes, N. M., Barnes, N. M., Costall, B., Horowitz, Z. P. & Naylor, R. J. *Brain Res.* (in the press).
14. Richardson, B. P., Engel, G., Donatsch, P. & Stadler, P. A. *Nature* **316**, 126–131 (1985).
15. Hoyer, D. T. *Pharmac. Sci.* **9**, 89–93 (1988).
16. Butler, A., Hill, J. M., Ireland, S. J., Jordan, C. C. & Tyers, M. B. *Br. J. Pharmac.* **94**, 397–412 (1988).
17. Smith, W. W., Sancilio, L. F., Owerla-Atepo, J. B., Naylor, R. J. & Lambert, L. L. *J. Pharm. Pharmac.* **40**, 301–302 (1988).
18. Neijt, H. C., Te Duits, I. J. & Vijverberg, H. P. M. *Neuropharmac.* **27**, 301–307 (1988).
19. Ireland, S. J. & Tyers, M. B. *Br. J. Pharmac.* **90**, 229–238 (1987).
20. Fozard, J. R. & Mobarak Ali, A. T. M. *Eur. J. Pharmac.* **49**, 109–112 (1978).

Cytoadherence of knobless *Plasmodium falciparum*-infected erythrocytes and its inhibition by a human monoclonal antibody

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RED blood cells infected with mature stages of the malaria parasite *Plasmodium falciparum* bind to the endothelial lining of capillaries and venules. This sequestration is important for the survival of the parasite but may have severe consequences for the host. For example, it is involved in the causation of cerebral malaria which carries 25% mortality^{1–5}. Knob-like protrusions present on the surface of infected erythrocytes have been considered necessary but not sufficient for this cytoadherence^{6–8}. Here we describe the adhesion to endothelial cells of infected erythrocytes which do not have knobs. A human monoclonal antibody (33G2) which was specific for an epitope containing regularly spaced dimers of

glutamic acid present in the repeated amino-acid sequences of some defined *P. falciparum* antigens^{9,10} was found to inhibit cytoadherence and may therefore be an important reagent for elucidating the molecular basis of parasite sequestration.

A bulk culture of the Palo Alto (PA) strain of *P. falciparum* bound poorly to the melanoma cell line C32 (100–200 infected red blood cells (RBC) per 100 melanoma cells). However, the number of binding RBC was significantly increased (2,000–3,000 RBC per 100 melanoma cells) after four consecutive adsorptions to melanoma cells and subsequent culture of the adherent infected RBC *in vitro* (Fig. 1). The cytoadherent RBC (C⁺) also bound to blood monocytes (1,000–1,200 RBC per 100 monocytes) and to endothelial cells from the human umbilical cord (100–3,400 RBC per 100 endothelial cells). The C⁺ phenotype was stable and was retained for at least eight months, although at a somewhat lower level (600–800 RBC per 100 melanoma cells).

Previous studies of cytoadherence by transmission electron microscopy (TEM) have shown that the points of close contact between infected RBC and endothelial cells were knobs^{1–5,7,8}. TEM showed that the C⁺ cells, as well as those of the original PA culture we investigated, lacked knobs (K[−]) (Fig. 2a), and that the adhesion sites between RBC and melanoma cells were close and short alignments of the plasma membranes (Fig. 2b). When probed in immunofluorescence with the mouse monoclonal antibody 89, which is specific for the knob-associated antigen HRP-1 (KAHRP)¹¹, the staining of K[−]C⁺ RBC was essentially negative.

Like the melanoma-binding of K⁺ cells^{6,12}, that of K[−] cells was trypsin-sensitive (binding ~45% of control at 100 µg ml^{−1}) and was inhibited by the monoclonal antibody (mAb) OKM5 directed against the antigen CD36 on platelets, endothelial cells, melanoma cells and monocytes^{13,14}. These results indicate that the molecular events involved in cytoadherence of K⁺ and K[−]

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cells are similar or identical. But further experiments are needed to settle this question and also to establish the possible involvement of thrombospondin^{2,15} in the cytoadherence of K⁻ cells.

Only one of five immunoglobulins (IgGs) from *P. falciparum* immune donors inhibited cytoadherence of K⁻ cells to melanoma cells (50% inhibition titre, 2 mg ml⁻¹), although all

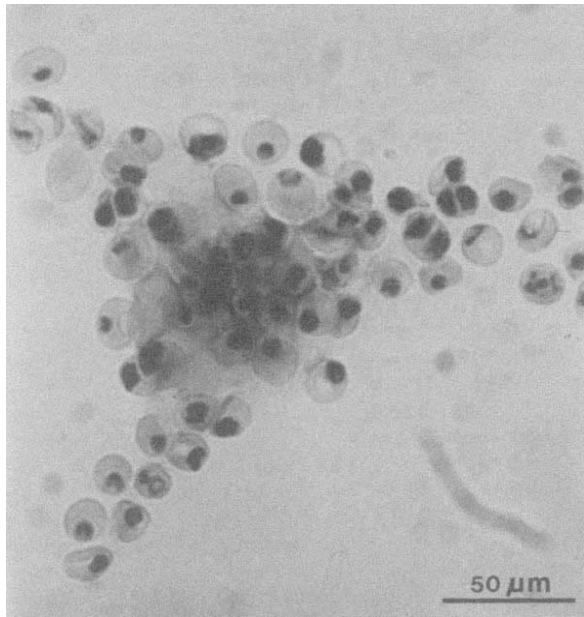


FIG. 1. Giemsa-stained *P. falciparum* RBC (Palo Alto) binding to melanoma cells (C32r).

METHODS. The cytoadherence assay was performed as described²⁵. Briefly, melanoma cells grown on cover slips were fixed with 1% formaldehyde in phosphate-buffered saline (PBS) and stored at 4 °C until used. C⁺ RBC (trophozoites/schizonts; 5–10% parasitaemia) were incubated with the melanoma cells at room temperature on a rotating platform for 1 h. Unbound RBC were flushed loose with PBS until only bound RBC remained. The coverslips were fixed with 1% glutaraldehyde in PBS, stained with Giemsa and examined in the light microscope.

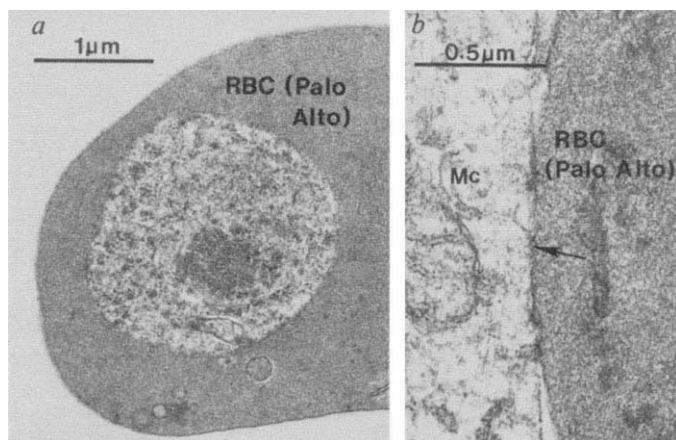


FIG. 2. *a*, Knobless *P. falciparum* RBC (Palo Alto) and *b*, binding (arrow) to a melanoma cell (Mc), as seen by transmission electron microscopy.

METHODS. Melanoma cells were grown in Petri dishes and fixed with 1% formaldehyde for 1 h. After immersion in PBS containing 0.2% BSA, they were scraped loose from the dishes. The cell suspensions were mixed with an equal volume of C⁺ RBC (5–10% parasitaemia) and incubated under gentle rotation for 1 h at room temperature. The RBC/melanoma conjugates were collected by sedimentation and washed by addition of fresh PBS until most of the unbound RBC were removed. The sediments were fixed for 1 h at room temperature with 2.5% glutaraldehyde in 0.05M PBS (pH 7.4) containing 4% sucrose. After fixation, embedding and sectioning was as described²⁶.

gave positive immunofluorescence with the surface of RBC containing late-stage parasites. But the human monoclonal antibody 33G2 (IgM, κ) prepared from a malaria-immune donor¹⁶, strongly inhibited the binding of the K⁻ cells in a dose-dependent manner (Fig. 3*b*). These results indicate either that the structures involved in cytoadherence of K⁻ cells are weakly immunogenic, or that the corresponding antigens display a strain diversity similar to that seen in the cytoadherence of K⁺ cells^{17,18}.

The inhibitory human mAb 33G2 both stains the surface of suspended (unfixed) K⁻C⁺-PA cells in immunofluorescence and agglutinates these cells, indicating that there is a surface expression of the corresponding epitope. Staining, agglutination and cytoadherence inhibition were also obtained with K⁺-cultures of the Gambian *P. falciparum* strain FCR-3. Interestingly, RBC infected with PA parasites depleted of C⁺ cells by repeated melanoma adsorptions were neither stained nor agglutinated by 33G2 and this was also true for K⁻C⁻ cells infected with the Tanzanian strain F32. Taken together, these results suggest that the epitope seen by the 33G2 antibody is associated with cytoadherence of both K⁺ and K⁻ RBC.

Antibody 33G2 is specific for a repeated epitope consisting of regularly spaced dimers of glutamic acid in antigen 332, a parasite-encoded polypeptide present in the membrane of RBC containing mature asexual stages of *P. falciparum*^{9,10}. So far no

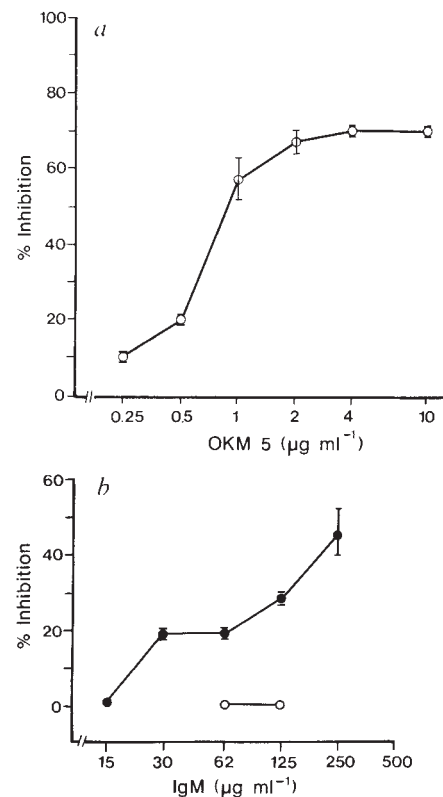


FIG. 3. Inhibition of cytoadherence by different antibodies. *a*, Inhibition by the monoclonal mouse antibody OKM5. *b*, Inhibition by the human monoclonal IgM antibody 33G2 (●). A human monoclonal IgM antibody specific for bacterial lipopolysaccharide (provided by Dr A. Rosén) was used as a control (○).

METHODS. *a*, Coverslips with formaldehyde-fixed melanoma cells (1% in PBS) were incubated with twofold dilutions of mAb OKM5 (0.25–10 μg ml⁻¹) for 30 min at room temperature. After three washes with PBS, C⁺ RBC (Palo Alto) were added to the melanoma cells and the binding assay was performed as described (Fig. 1). The number of bound RBC per 100 melanoma cells was counted and expressed as the percentage of bound cells. *b*, Aliquots (0.5 ml) of concanavalin A-purified 33G2 (ref. 16) or of the control antibody were added to the pellets (15–250 μg ml⁻¹) and incubated for 30 min at 37 °C (total volume ~0.2 ml). One ml of PBS was added to each tube after incubation and the mixtures were then applied to the melanoma cells on coverslips. The binding assay and calculations were as in *a*. In each case, the mean and range % inhibition from two experiments are shown.

differences have been seen when lysates of C⁺ or C⁻ parasites separated by SDS-PAGE were probed with 33G2 in immunoblotting, and the possible relationship of antigen 332 to any of the polypeptides previously postulated to be associated with cytoadherence¹⁹⁻²¹ is unknown. Antibody 33G2 cross-reacts with similar sequences of genetically unrelated *P. falciparum* antigens such as Pf155/RESA^{9,16}, which cannot be implicated in cytoadherence¹⁹. But it also binds to Pf11.1 (refs 9, 22 and 23), a high-molecular weight polypeptide which may play a part in this process¹⁹. Thus it is not clear at present whether it is antigen 332, Pf11.1 or some other cross-reacting antigen which is responsible for cytoadherence-inhibition by antibody 33G2.

The present findings apparently conflict with earlier results, in so far as knobs at the surface of erythrocytes infected with asexual *P. falciparum* parasites have been considered necessary for cytoadherence. Sequestration of immature *P. falciparum* gametocytes has, however, been shown to occur independently of knob protrusions²⁴. Similarly, sequestration of RBC containing asexual stages in other malarial (*P. knowlesi*, *P. berghei*, *P. chabaudi*) may also occur in the absence of knobs, whereas in other malarial species (*P. malariae*, *P. brasilianum*), infected RBC express knobs but do not cytoadhere¹⁹. Thus the presence of knobs seems necessary for cytoadherence of some parasite populations but not others. In any event, as the human monoclonal antibody 33G2 efficiently inhibits cytoadherence it may become a useful adjunct in the treatment or prophylaxis of severe *P. falciparum* malaria. □

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1. Aikawa, M. *Am. J. trop. Med. Hyg.* **39**, 3-10 (1988).
2. MacPherson, G. G., Warrell, M. J., White, N. J., Looareesuwan, S. & Warrell, D. A. *Am. J. Path.* **119**, 385-401 (1985).
3. Oo, M. M. *et al. J. Neuropath. exp. Neurol.* **46**, 223-231 (1987).
4. Luse, S. A. & Miller, L. H. *Am. J. trop. Med. Hyg.* **20**, 655-660 (1971).
5. Trager, W., Rudzinska, M. A. & Bradbury, P. C. *Bull. Wild Hth Org.* **35**, 883-885 (1968).
6. David, P. H., Hommel, M., Miller, L. H., Udeinya, I. J. & Oligino, L. D. *Proc. natn. Acad. Sci. U.S.A.* **80**, 5075-5079 (1983).
7. Raventos-Suarez, C., Kaul, D. K., Macaluso, F. & Nagel, R. L. *Proc. natn. Acad. Sci. U.S.A.* **82**, 3829-3833 (1985).
8. Udeinya, I. J., Schmidt, J. A., Aikawa, M., Miller, L. H. & Green, I. *Science* **213**, 555-557 (1981).
9. Mattei, D. *et al. Parasite Immun.* **11**, 15-30 (1989).
10. Udomsangpetch, R. *et al. J. Immun.* (in the press).
11. Taylor, D. W. *et al. Molec. biochem. Parasit.* **25**, 165-174 (1987).
12. Leech, J. H., Barnwell, J. W., Miller, L. H. & Howard, R. J. *J. exp. Med.* **159**, 1567-1575 (1984).
13. Barnwell, J. W., Ockenhouse, C. F. & Knowles, D. M. *J. Immun.* **135**, 3494-3497 (1985).
14. Ockenhouse, C. F. & Chulay, J. D. *J. infect. Dis.* **157**, 584-588 (1988).
15. Roberts, D. D. *et al. Nature* **318**, 64-66 (1985).
16. Udomsangpetch, R. *et al. Science* **231**, 57-59 (1986).
17. Udeinya, I. J., Miller, L. H., McGregor, I. A. & Jensen, J. B. *Nature* **303**, 429-431 (1983).
18. Singh, B. *et al. Clin. exp. Immun.* **72**, 145-150 (1988).
19. Howard, R. J. *Prog. Allergy* **41**, 98-147 (1988).
20. Howard, R. J. *et al. Molec. Biochem. Parasit.* **27**, 207-224 (1988).
21. Magowan, C., Wollish, W., Anderson, L. & Leech, J. *J. exp. Med.* **168**, 1307-1320 (1988).
22. Koenen, M. *et al. Nature* **311**, 382-385 (1984).
23. Scherf, A. *et al. EMBO J.* **7**, 1129-1137 (1988).
24. Smalley, M. E., Abdalla, S. & Brown, J. *Trans. R. Soc. trop. Med. Hyg.* **75**, 103-105 (1980).
25. Udeinya, I. J., Graves, P. M., Carter, R., Aikawa, M. & Miller, L. H. *Expl. Parasit.* **56**, 207-214 (1983).
26. Aikawa, M., Uni, Y., Andrusis, A. T. & Howard, R. J. *Am. J. trop. Med. Hyg.* **35**, 30-36 (1986).

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A novel MHC class II epitope expressed in thymic medulla but not cortex

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THE repertoire of receptors expressed by peripheral T cells is the result of two selective events that occur during intrathymic development. Positive selection expands cells able to recognize foreign peptides presented by self MHC molecules, and negative selection eliminates cells reactive to self MHC molecules and associated self peptides¹⁻⁴. Chimaera studies suggest that, at least in the case of T cells recognizing MHC class II, interaction with thymic cortical epithelial cells is responsible for the former⁵, whereas thymic medullary cells, of bone marrow origin, mediate the latter^{3,6-8}. This view of thymic development is supported by recent morphometric analyses⁹, showing that autoreactive cells are found in thymic cortex but not medulla. Although numerous^{1-3,10-12} studies have shown that MHC class II molecules are expressed in both sites, none provides any explanation for the differential selection of T cells that is observed. Here, we describe a novel MHC class II epitope which is found on cells in thymic medulla but not cortex. The antibody to this epitope reacts with about 10% of class II molecules on B cells and may be recognizing a self peptide-MHC complex. These results provide the first evidence for differential expression of class II epitopes in different tissues and are compatible with the hypothesis that different ligands^{2,13-16},

rather than different affinity thresholds for the same ligand^{17,18}, are involved in positive and negative selection of the T-cell repertoire.

The monoclonal antibody used in this study, Y-Ae, was produced by immunizing class II incompatible mice with lipopolysaccharide activated spleen cells (Fig. 1). The antibody specifically reacts with splenic B cells from strains which express I-A^b(A_αA_β) and I-E^b(E_αE_β) surface complexes, such as donor strain B10.A(5R) (Fig. 1a, b). It does not react with I-A molecules alone, as cells which express surface I-A^b molecules, including strain B10.A(18R) spleen cells (Fig. 1a) and L cells transfected with A_α and A_β genes (Fig. 1c), are Y-Ae-negative. The antibody also does not react with I-E molecules, as several cell types that express surface I-E^b complexes, including L cells transfected with E_α^{d/k} and E_β^b genes (Fig. 1d) and (B6.C-H-2^{bm-12} × B10.TFR5)F1 spleen cells (Fig. 1h), are type Y-Ae-negative. However, both I-A and I-E genes influence the Y-Ae determinant. Formal proof that the E_α gene or surface E_αE_β complexes are involved comes from the observation that Y-Ae reacts with spleen cells from B6-E_α^d transgenic strain 107.1 (Fig. 1f) but not progenitor B6 (Fig. 1e). Studies with A_β mutant strain B6.C-H-2^{bm-12} provide clear evidence that at least the A_β gene also influences Y-Ae activity, as shown by reactivity of the antibody with (B6 × BALB/c)F1 (Fig. 1g) but not (B6.C-H-2^{bm-12} × B10.TFR5)F1 (Fig. 1h).

Evidence that the Y-Ae antibody binds to a modified form of I-A complexes comes from several sources. First, the antibody precipitates only mature forms of A_α^b and A_β^b chains from biosynthetically labelled spleen cell lysates (Fig. 2). No E_α^b chains, E_β^b chains, or invariant (Ii) polypeptides were observed in these immunoprecipitates. This formally excludes the possibility that the antibody reacts with mixed isotype E_α^bA_β^b dimers. Previous study showed that E_α chains cannot pair with A_β^b chains¹⁹. Failure to detect Ii chains suggests that the antibody reacts only with surface forms of class II molecules²⁰. Second, several monoclonal antibodies^{21,22} that react with sites influenced by α-helical residues on A_α(Y-3P, Y-248) or A_β(Y-238) chains inhibit binding of Y-Ae to the lymphocyte surface, whereas monoclonal antibodies reactive with other parts of I-A molecules (Y-219) or with I-E molecules (Y-17, 14-4-4) failed

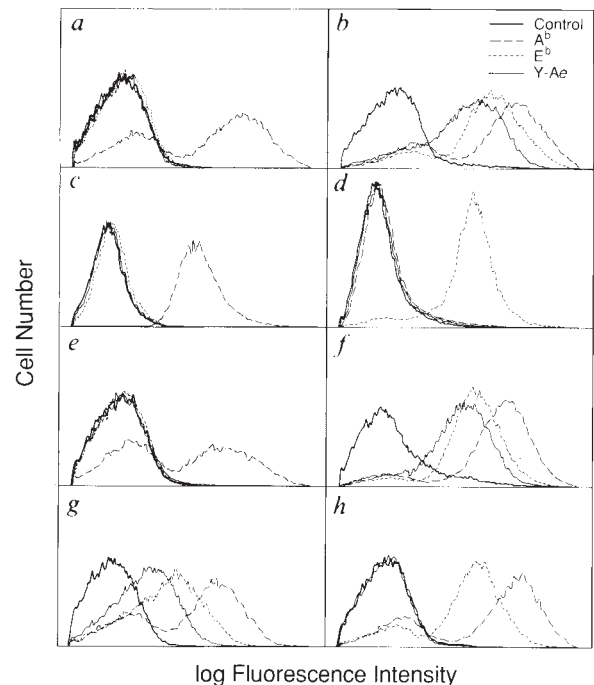
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to block (data not shown). Third, and most important, Y-Ae binds to about 10% of surface I-A molecules, as judged by competitive inhibition and quantitative binding analyses (Fig. 3). Taken together, these studies demonstrate that Y-Ae binds to a novel B-cell epitope on a subset of I-A^b molecules in strains expressing surface I-E^b molecules.

FIG. 1 The Y-Ae antibody does not react with conventional I-A and I-E molecules, yet both I-A and I-E genes influence the Y-Ae determinant. Immunofluorescent analysis was carried out using the following biotin-conjugated monoclonal antibodies and cells. Control (—): anti-I-A^b(A^b_αA^b_β), γ2b, 11.5.2 (ref. 24); A^b(- - -): anti-I-A^b(A^b_αA^b_β), γ2a, Y-3P (ref. 25); E^b(· · · · ·): anti-I-E^b(E^b_αE^b_β), γ2b, Y-17 (ref. 26); and Y-Ae (—): (B10.D2 × B10.MBR)F1 anti-B10.A(5R) fused with Ag-8 and cloned by limiting dilution, γ2b. Panel a, B10.A(18R) spleen cells, which express A^b_αA^b_β surface molecules and E^b_α cytoplasmic chains, but not E^b_α chains. b, B10.A(5R) spleen cells which express A^b_αA^b_β and E^b_αE^b_β surface molecules. c and d, L cells transfected with A^b_α and A^b_β genes (c) or with E^b_α and E^b_β genes (d), gifts of Dr R. Germain^{27,28}. e, B6 spleen cells, which possess a nonfunctional E^b_α gene and express A^b_αA^b_β surface complexes and E^b_α cytoplasmic chains. f, B6-E^b_α transgenic strain 107-1 spleen cells, which possess a functional E^b_α transgene and express A^b_αA^b_β and E^b_αE^b_β surface molecules in a normal manner. g, (B6 × BALB/c)F1 spleen cells which express A^b_αA^b_β and E^b_αE^b_β surface molecules. h, (B6.C-H-2^{bm-12} × B10.TFR5)F1 spleen cells, which express mutant A^b_αA^b_β and normal E^b_αE^b_β surface molecules. Similar results were obtained with (B6 × A.TFR5)F1(Y-Ae⁺) and (B6.C-H-2^{bm-12} × A.TFR5)F1 (Y-Ae⁻) spleen cells (data not shown). Relevant difference between both sets of F1 hybrids is in the A_β chain, where A^b_β differs from A^{bm-12}_β at residues 67, 70 and 71 (ref. 29). In summary, the Y-Ae positive strains tested include: B10.A(5R), B10.A(3R), B10.BAR23, B10.BAR26, B10.BAR27, all 5R-like strains which express surface A^b_αA^b_β and E^b_αE^b_β complexes³⁰, and B6-E^b_α strain 107-1. The Y-Ae negative strains tested include: B6, B10, BALB.B, A.BY, B10.D2, BALB/c, B10.M, A.CA, B10.BR, BALB.K, C3H, B10.F(13R), B10.G, B10.RIII, B10.S, A.SW, B10.PL, B10.SM, B10.A, A, B10.A(2R), B10.A(4R), B10.A(18R), B10.MBR, B10.GD, A.TFR5, B10.TFR5, B10.HTT, B10.S(9R), B6.C-H-2^{bm-12}, and B6-E^b_α transgenic strain 36-5.

METHODS. Biotin-conjugated antibodies were added to spleen cell or L-cell suspensions for 30 min at 4 °C in phosphate-buffered saline containing 2% fetal calf serum, 0.1% sodium azide, and 1 mg ml⁻¹ normal mouse immunoglobulin to inhibit binding to Fc receptors (staining buffer). All antibodies were used at plateau staining concentrations. After washing with

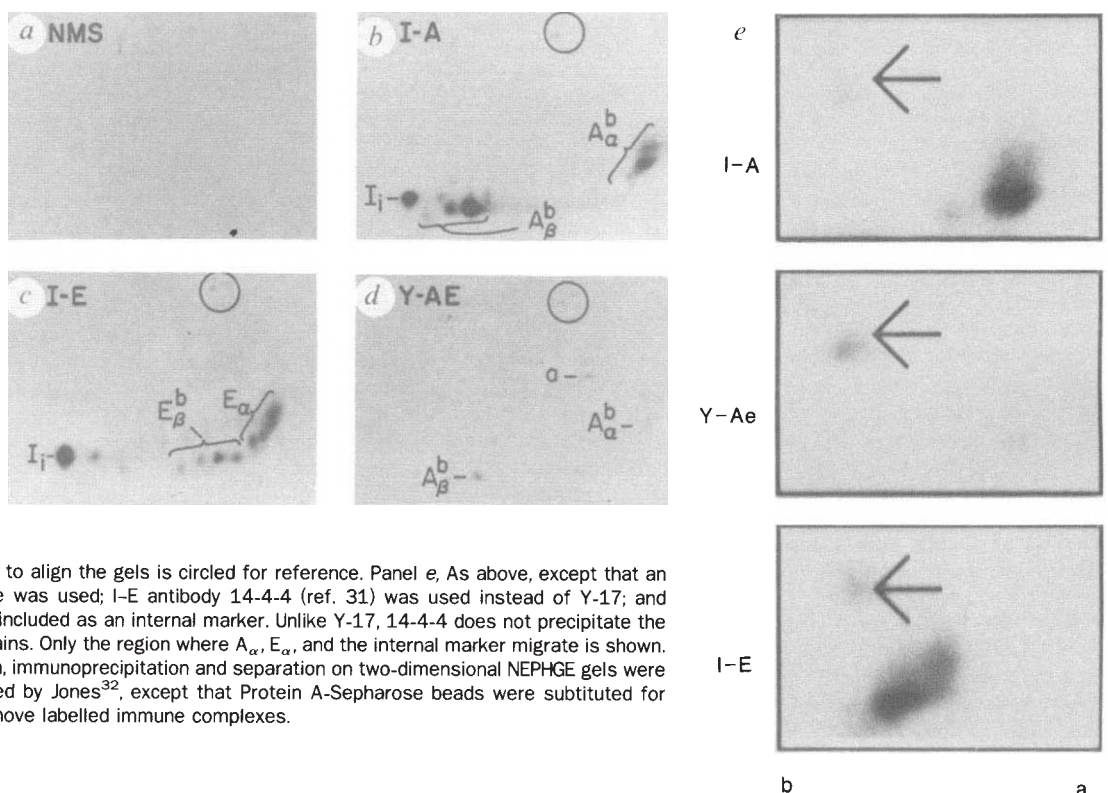
The epitope detected by Y-Ae on B cells is also strongly expressed on cells in the thymic medulla, but is not expressed on epithelial cells in the thymic cortex (Fig. 4). With both the B6-E^b_α transgenic strain 107-1 and strain B10.A(5R) (data not shown), anti-I-A (Fig. 4a) and anti-I-E (Fig. 4b) antibodies give typical confluent staining of the medulla and reticular



staining buffer, cells were stained with FITC-Avidin for 20 min at 4 °C. Washed cells (20,000 per sample) were analysed on a FACS 440 (panels a, b, e-h) or a FACScan analyser (panels c, d). Cell number is displayed against a 3.7 log unit axis of fluorescence intensity.

FIG. 2 The Y-Ae antibody reacts with mature forms of A_α and A_β chains. Panels a-d, two dimensional SDS-PAGE analysis of [³⁵S]-methionine-labelled B10.A(5R) spleen cell lysates immunoprecipitated with normal mouse serum, anti-I-A (Y-3P), anti-I-E (Y-17) or Y-Ae. Note the identity but different intensity of the spots precipitated from the same lysate by Y-Ae and the major A_α and A_β spots precipitated by Y-3P; their distinction from all I-E spots precipitated by Y-17; and the absence of invariant chain (Ii) in the Y-Ae precipitate. A faint spot labelled (a) is actin. In the three immunoprecipitates, an invariant triplet of spots used to align the gels is circled for reference. Panel e, As above, except that an independently prepared lysate was used; I-E antibody 14-4-4 (ref. 31) was used instead of Y-17; and ¹⁴C-labelled OVA (arrow) was included as an internal marker. Unlike Y-17, 14-4-4 does not precipitate the most immature form of E_α chains. Only the region where A_α, E_α, and the internal marker migrate is shown.

METHODS. Labelling, extraction, immunoprecipitation and separation on two-dimensional NEPHGE gels were carried out exactly as specified by Jones³², except that Protein A-Sepharose beads were substituted for Staph A (Cowan strain) to remove labelled immune complexes.



staining of the cortex, whereas Y-Ae (Fig. 4c) reacts only with cells in the medulla. In contrast, the Y-Ae antibody does not react with any cells in the B6- E_α^d transgenic strain 36-5 (Fig. 4f), which expresses I-A^b molecules normally (Fig. 4d) and I-E^b molecules on thymic epithelial cells only (Fig. 4e). This suggests that the Y-Ae epitope is expressed on bone marrow-derived cells in the thymic medulla.

Although it is clear that the Y-Ae antibody reacts with a subset of I-A molecules, and that an I-E gene influences the determinant recognized, the nature of the unique epitope detected is not yet known. We favour the interpretation that the antibody may be much like a T-cell receptor, detecting a self peptide:I-A complex. The most likely source of this peptide is the E_α or E_β chain. If the self-peptide:MHC model is correct, the antibody

will be extremely useful in analysing antigen processing at the molecular and ultrastructural levels. A second possibility is that the antibody reacts with a complex between I-A molecules and some intact protein whose expression is influenced by I-E molecules, such as the unknown products of *Mls* loci²³. Other interpretations are also possible. To date, neither a processed peptide nor a third polypeptide chain has been detected by immunoprecipitation (Fig. 2).

The finding that a unique class II epitope is expressed on peripheral B cells and cells in the thymic medulla but not cortex is the first direct evidence that MHC molecules differ in conformation in a tissue-specific fashion. These results raise the possibility that the ligands involved in positive and negative selection of the T-cell repertoire may actually be different. If thymic

FIG. 3 The Y-Ae antibody reacts with a subset of I-A^b molecules on B10.A(5R) B cells. Panel a, Unlabelled I-A^b antibody Y-3P completely blocks binding of ¹²⁵I-labelled Y-Ae antibody to the B cell surface. b, Unlabelled Y-Ae partially inhibits binding of ¹²⁵I-labelled Y-3P. c, At saturation, the Y-Ae antibody binds at about 10% of the level obtained with Y-3P. d, The Y-Ae antibody binds with an avidity about 10-fold greater than that of Y-3P, as judged by Scatchard analysis.

METHODS. T cells were depleted with anti-Thy-1 plus complement. Antibodies were ¹²⁵I-labelled, and binding assays carried out and analysed as previously described³³, except that instead of washing extensively, cells were separated from unbound antibody by a single passage over oil-stop³⁴.

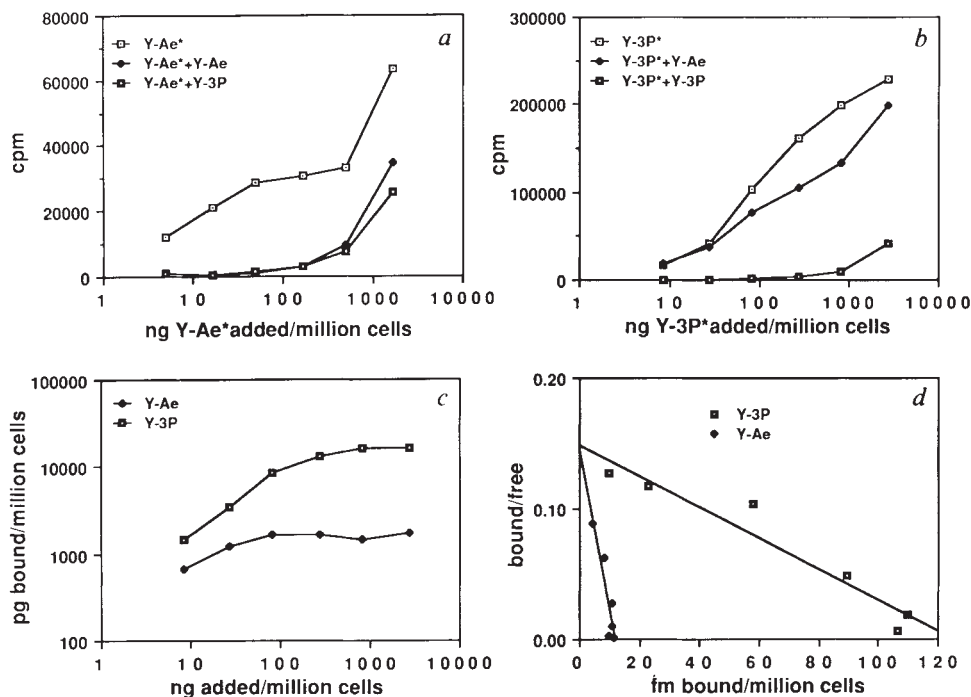
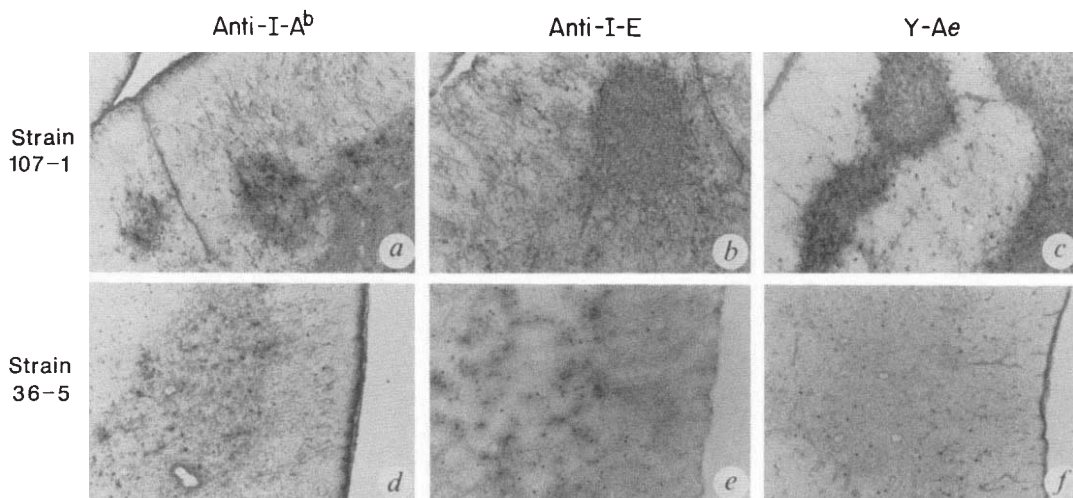


FIG. 4 The Y-Ae antibody reacts with cells in thymic medulla but not cortex. Immunoperoxidase staining of frozen thymus sections shows the distribution of I-A (panels a, d), I-E (panels b, e), and the determinant detected by Y-Ae (panels c, f) in different B6- E_α^d transgenic mice. Strain 107-1 (panels a, c) expresses I-A molecules normally, whereas in strain 36-5 (panels d-f), I-E molecules are expressed only on thymic epithelium (compare panels b and e). Y-Ae stains the thymic medulla in strain 107-1, but does not stain thymic cortex in either strain, despite the presence of strong staining with both anti-I-A and anti-I-E antibodies. This result is independent of the concentration of antibody used, and was also obtained with B10.A(5R) mice (data not shown). Neither anti-I-E nor Y-Ae antibodies stain above the background seen in panel f with thymus sections from progenitor B6 mice or B6- E_α^d transgenic mice expressing I-E molecules only in the pancreas (data not shown).

METHODS. Cryostat sections (10 μ m) of thymus from strain 107-1 or strain



36-5 were fixed in acetone and hydrated in PBS. Primary biotinylated antibodies, diluted in PBS with 30 mg ml⁻¹ BSA, were layered over the sections for 30 min. Sections were washed in phosphate buffered saline, and the bound biotinylated antibodies were detected using avidin-HRP (ABC-Elite, Vector labs) with DAB as the chromogen. Magnification factor: ~60-100 $\times$. The derivation and characterization of the transgenic mice is described in refs 10 and 11.

medullary cells are involved in negative selection by clonal deletion¹⁻⁴, then one can envisage T-cell development as occurring sequentially, with cells being positively selected for recognition of one set of class II epitopes on thymic epithelium, followed by the deletion of cells cross-reactive with unique epitopes found in the thymic medulla and on peripheral cells. □

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1. Janeway, C. A., Jr *Nature* **335**, 208-210 (1988).
2. Marrack, P. & Kappler, J. *Immun. Today* **9**, 308-315 (1988).
3. Sprent, J., Lo, D., Gao, E.-K. & Ron, Y. *Immunol. Rev.* **101**, 171-190 (1988).
4. von Boehmer, H., Teh, H. S. & Kisielow, P. *Immun. Today* **10**, 61-66 (1989).
5. Lo, D. & Sprent, J. *Nature* **319**, 672-674 (1986).
6. Jenkinson, E. J., Jhittay, P., Kingston, R. & Owen, J. J. T. *Transplantation* **39**, 331-342 (1985).
7. von Boehmer, H. & Schubiger, K. *Eur. J. Immun.* **14**, 1048-1052 (1984).
8. Marrack, P. *et al. Cell* **53**, 627-634 (1988).
9. Hengartner, H. *et al. Nature* **336**, 388-390 (1988).
10. Widera, G. *et al. Cell* **51**, 175-187 (1987).
11. Lo, D. *et al. Cell* **53**, 159-168 (1988).
12. van Ewijk, W. *et al. Cell* **53**, 357-370 (1988).
13. Janeway, C. A., Jr. *Immun. Today* **3**, 261-265 (1982).
14. Singer, A., Mizuuchi, T., Munitz, T. I. & Gress, R. E. *Prog. Immun.* **6**, 60-66 (1986).
15. Claverie, J. M. & Kourilsky, P. *Ann. Inst. Pasteur Immun.* **137D**, 425-437 (1986).

16. Kappler, J. W., Staerz, U., White, J. & Marrack, P. *Nature* **332**, 35-40 (1988).
17. Janeway, C. A., Jr, Binz, H. & Wigzell, H. *Scand. J. Immun.* **5**, 993-1001 (1976).
18. Blanden, R. V. & Ada, G. L. *Scand. J. Immun.* **7**, 181-190 (1978).
19. Sant, A. J., Braunstein, N. S. & Germain, R. N. *Proc. natn. Acad. Sci. U.S.A.* **84**, 8065-8069 (1987).
20. Sung, E. & Jones, P. *Molec. Immun.* **18**, 899-913 (1981).
21. Buerstedde, J. M. *et al. J. exp. Med.* **167**, 473-487 (1988).
22. Landais, D. *et al. Cell* **47**, 173-181 (1986).
23. Janeway, C. A. Jr *et al. Immunol. Rev.* **107**, 61 (1989).
24. Oi, V. T., Jones, P. P., Goding, J. W., Herzenberg, L. A. & Herzenberg, L. A. *Curr. Topics Microbiol. Immun.* **81**, 115-129 (1978).
25. Janeway, C. A. Jr *et al. J. Immun.* **132**, 662-668 (1984).
26. Lerner, E. A. *et al. J. exp. med.* **152**, 1085-1101 (1980).
27. Ronchese, F., Brown, M. A. & Germain, R. N. *J. Immun.* **139**, 629-638 (1987).
28. Ronchese, F., Schwartz, R. H. & Germain, R. N. *Nature* **329**, 254-256 (1987).
29. McIntyre, K. R. & Siedman, J. G. *Nature* **308**, 551-553 (1984).
30. Murphy, D. B. *Mouse News Lett.* **74**, 73-75 (1986).
31. Ozato, K., Mayer, N. & Sachs, D. H. *J. Immun.* **124**, 533-540 (1980).
32. Jones, P. P. in *Selected Meth. Cell. Immun.* (eds Mishell, B. P. & Shigii, S. P.) 398-440 (Freeman, San Francisco, 1980).
33. Rojo, J. & Janeway, C. A. Jr *J. Immun.* **140**, 1081-1089 (1988).
34. Greenbaum, L. A. *et al. J. Immun.* **140**, 1555-1560 (1988).

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Incubation time for AIDS from French transfusion-associated cases

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ALTHOUGH incubation time is a key parameter of the epidemiology of AIDS, statistical estimates based on transfusion-associated AIDS cases have, up to now, used only the single dataset provided by the AIDS program of the Centers for Disease Control (CDC) in Atlanta. Using a new dataset provided by the Direction Générale de la Santé (DGS), of the French Ministry of Health¹, we estimate the mean incubation time for AIDS (median in brackets) to be 5.3 years (5.3 years) with a 90% confidence interval ranging from 4.4 to 8.9 years (4.4 to 8.8 years), when a Weibull distribution is postulated for incubation time. The previously encountered problem of very large confidence intervals (range larger than 100 years)², is not observed, indicating that an accurate estimate for mean incubation time will be obtainable in the near future.

In France, the country with the second highest incidence of

AIDS cases³, 259 transfusion-associated cases, diagnosed before January 1988 by WHO criteria⁴, had been reported to the DGS by 25 March 1988. (Reporting AIDS cases to the DGS is mandatory in France.) Each case was individually reviewed by an epidemiologist to verify the quality of the data reported. Ninety-two cases associated with multiple transfusion, single transfusion without knowledge of the time of transfusion or other major transmission risk factors (homosexual or bisexual men, haemophiliacs, intravenous drug users) were eliminated, leading to a dataset of 167 subjects¹. Due to the age-dependence of incubation time^{5,6}, the analysis was further restricted to all subjects 13 years of age or older. Thus, the final dataset used to estimate mean and median incubation times for AIDS, and to obtain confidence intervals for these two parameters, comprised 148 subjects.

To analyse the data, we used the classical maximum likelihood method for which two derivations of the likelihood function that are applicable to the present problem have been described. The approach described by Medley *et al.*^{5,6} is conditional on only N , the total number of transfusion-associated cases observed and allows for an exponential growth in infected transfusions before 1985 and a constant number since then. The method used by Lui *et al.*^{7,8} is conditional on N and on the times $t_1, t_2, \dots, t_N$ of observed infections, and provides a truncated likelihood. As both methods lead to a valid conditional likelihood⁹ and as the derivation of confidence intervals is easier with the approach described by Lui *et al.*, the latter method was selected. A Weibull or a γ -distribution for incubation time were postulated as in several previous works^{2,5-8}. To derive confidence intervals for mean and median incubation times for AIDS, the

TABLE 1 Expected and observed numbers of transfusion-associated AIDS cases by year of transfusion and year of diagnosis

Year of diagnosis	Year of transfusion									Total
	1978	1979	1980	1981	1982	1983	1984	1985		
1982	0.1	0.0	0.1	0.0	0.0	(expected)	1.0			0.2
	1	0	0	0	0	(observed)				1
1983	0.2	0.0	0.3	0.3	0.1	0.0				0.9
	0	0	0	1	0	0				1
1984	0.2	0.0	0.5	0.8	1.4	0.4	0.0			3.3
	0	0	0	0	3	2	0			5
1985	0.2	0.0	0.7	1.5	3.8	3.5	1.5	0.0		11.2
	0	0	1	1	2	2	3	0		9
1986	0.1	0.0	0.8	2.1	7.1	9.9	14.4	2.5		36.9
	0	0	2	2	3	17	18	3		45
1987	0.0	0.0	0.6	2.2	9.6	18.2	40.1	24.5		95.2
	0	0	0	3	14	11	35	24		87

The expected numbers are calculated using the Weibull distribution for incubation time.

likelihood ratio statistics (LRS), accepted as the best method in cases where data are truncated¹⁰, was used. Using a Weibull distribution, our point estimate for the mean (median) incubation time is 5.3 years (5.3 years). The 90% confidence interval is 4.4–8.9 years (4.4–8.8 years). The corresponding results with a γ -distribution are 6.9 years for the mean (6.4 years for the median) with a 90% confidence interval ranging from 4.9–19.0 years (4.6–17.1 years). An adjustment of the Weibull distribution to the data is presented in Table 1. Not surprisingly, the χ^2 value of comparison of the expected and observed values is not significant ($\chi^2 = 7.0$, 3 d.f.). A similar fit was observed with a γ -distribution, for the log of the likelihood value (see legend to Fig. 1), even if the confidence intervals for this distribution seem to be larger.

To compare our results with the most recent results obtained from the US dataset (by the method of Medley *et al.*¹¹), the US data were analysed using the method described above^{7,8}. The 543 cases reported to CDC by 8 April 1988, on the same selection criteria as for the French dataset, were included in the analysis. Our estimate of the mean, as compared with the last published analysis from Medley *et al.*¹¹, was 7.3 years (90% confidence interval 5.7–11.7 years) versus 7.6 years, with a Weibull distribution and 9.8 years (90% confidence interval 7.1–19.0 years) versus 24.1 years with a γ -distribution. A large discrepancy between the results obtained by the two different methods is observed only when a γ -distribution is postulated. This distribution, however, was rejected by Medley *et al.*¹¹, due to the very large annual rate of infective transfusions after 1985 that is predicted with their model with the γ -assumption. Moreover, the same authors now estimate¹² the mean incubation time at 14.3 years (γ -distribution), based on the April 1988 CDC data analysis. Unfortunately the authors do not comment on the sharp decrease (around 10 years) in their estimate.

The sizes of our confidence intervals seem relatively small compared with those reported by Kalbfleisch *et al.* (4.6, ∞ ; ref. 2). Our explanation is that the precision of the estimate of the mean (or median) incubation time is related to the duration of the follow-up rather than to the number of subjects. To investigate this hypothesis, the French and US cases diagnosed before 1 January 1987 (61 and 388 cases, respectively) were analysed

using the Weibull distribution. This led, respectively, to a 90% confidence interval for the mean of (4.4, ∞) and (5.7, ∞).

In conclusion, when assuming a Weibull distribution for incubation time, the mean or median incubation time estimated from the French dataset, range from 4.4–8.9 years (90% confidence intervals). The evolution of the range of confidence intervals for the mean incubation time over the past year, clearly shows that reasonably narrow confidence intervals will be obtainable in the near future $\square$

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1. Reidboym, M., Laporte, A. & Brunet, J. B. *Bull. Epidemiologique Hebdomadaire* **22**, 85–86 (1988).
2. Kalbfleisch, J. D. & Lawless, J. F. *Nature* **333**, 504–505 (1988).
3. WHO *Wkly Epidemiol. Rec.* **63**, 309–310 (1988).
4. WHO *Wkly Epidemiol. Rec.* **63**, 1–7 (1988).
5. Medley, G. F., Anderson, R. M., Cox, D. R. & Billard, L. *Nature* **328**, 719–721 (1987).
6. Medley, G. F., Billard, L., Cox, D. R. & Anderson, R. M. *Proc. R. Soc. B* **233**, 367–377 (1988).
7. Lui, K. J. *et al. Proc. natn. Acad. Sci. U.S.A.* **83**, 3051–3055 (1986).
8. Lui, K. J., Peterman, T. A., Lawrence, D. N. & Allen J. R. *Statist. Med.* **7**, 395–401 (1988).
9. Kalbfleisch, J. D., Lawless, J. F. *J. R. Statist. Soc. A* **151**, 47–48 (1988).
10. Kalbfleisch, J. D. & Prentice, R. L. *The Statistical Analysis of Failure Time Data* (Wiley, New York, 1980).
11. Medley, G. F., Anderson, R. M., Cox, D. R. & Billard, L. *Nature* **333**, 505 (1988).
12. Anderson, R. M. & Medley, G. F. *AIDS* **2** (Suppl. 1), S57–S63 (1988).

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Paramyxovirus SV5 and multiple sclerosis

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MULTIPLE sclerosis is commonly associated with a local humoral immune response within the central nervous system. A hallmark of this intrathecal response is the presence of electrophoretically demonstrable oligoclonal bands of IgG in the cerebrospinal fluid (CSF) of up to 95% of patients^{1,2}. Observations indicating that a major part of the CSF IgG in some patients may represent antibodies to SV5, a simian virus closely related to human parainfluenza type 2 virus, were recently reported by Goswami *et al.*³. We have studied thirty patients with multiple sclerosis, but although we find intrathecal synthesis of IgG antibodies reacting with SV5 in seven of these, the antibodies were not associated with oligoclonal CSF IgG bands and could in each case be explained as potentially cross-reacting antibodies to other paramyxoviruses known to be human pathogens. We have therefore been unable to confirm that SV5 may be a major intrathecal immunogen in multiple sclerosis.

Sera and CSF from thirty multiple sclerosis (MS) patients were analysed for IgG antibodies to SV5 and seven other viral antigens using an imprint immuno-fixation (IIF)⁴ technique. In agreement with other reports^{5–7}, intrathecal antibody responses to one or more viruses were observed in all thirty patients, seven of whom had intrathecal synthesis of antibodies reacting with SV5 (Fig. 1, Table 1). No evidence of intrathecal SV5 antibody synthesis was observed in six patients with defined neuroinfectious diseases (SSPE, HSV encephalitis, VSV encephalitis, tuberculous meningitis, Lyme neuroborreliosis, neurosyphilis) or in ten patients with neuroinflammatory diseases of undetermined aetiology.

Oligoclonal IgG bands were demonstrated by agarose gel electrophoresis and by IIF in the CSF from twenty-nine of the MS patients, including six of the seven with intrathecal SV5 antibody synthesis (Table 2). But the patterns of these bands did not correlate with those of intrathecally synthesized anti-

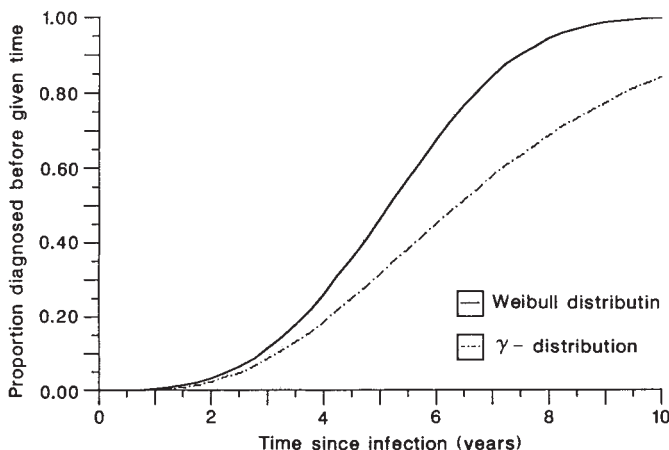


FIG. 1 The estimated incubation time described by Weibull distribution or γ -distribution shown as a proportion of transfusion-associated AIDS cases diagnosed before a given point in time. The 148 patients, 13 years of age or older, diagnosed before 1 January 1988 and reported to DGS by 25 March 1988 were included in the analysis. The density function according to the Weibull distribution is $pq^p x^{p-1} e^{-(qx)^p}$, where p and q are parameters, and x is the time between transfusion and diagnosis; the fitted parameters are $p = 3.23$ and $q = 0.17$ and the log likelihood is -509.203 . The γ -distribution has the density function $q^p x^{p-1} e^{-qx} / \Gamma(p)$, the fitted parameters are $p = 4.47$ and $q = 0.65$ and the log likelihood is -508.596 . The first parts of the curves were adjusted on the DGS dataset for up to 6.4 years (observed data) and then extrapolated from the model of the density function.

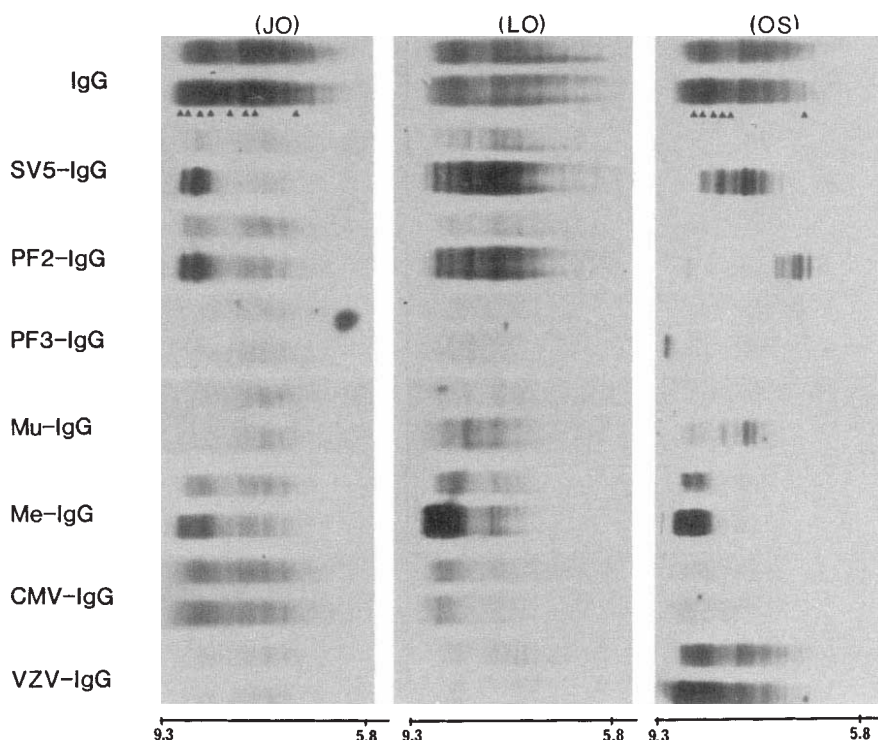


FIG.1 Imprint immunofixation (IIF) of total IgG and of IgG antibodies to SV5, parainfluenza (PF) viruses 2 and 3, mumps (Mu), measles (Me), varicella zoster (VZV) and cytomegalovirus (CMV) in electrofocussed (pH 3.5–9.5) pairs of serum and CSF from three patients with MS. Individual autoradiographs show serum above and CSF below. Note intrathecal synthesis of antibodies to SV5 and other viruses in each of the three patients (for criteria, see below) and of oligoclonal IgG bands (triangles) in two of the patients.

METHODS. SV5 (LN strain), PF2 (Greer strain), PF3 (C 243 strain) and mumps (Enders strain) cell pack antigens were prepared from infected Vero (African green monkey kidney) cell cultures. The infected cells were suspended in phosphate buffered saline at a concentration of ~10% (v/v), sonicated, and clarified by centrifugation (1,000 g for 5 min). Control antigen was prepared from non-infected Vero cells in the same way. Measles, HSV, VZV and CMV antigens were prepared as described previously⁷. Sera and concentrated CSF were adjusted to an IgG concentration of 2 g l⁻¹. The procedure used for IIF has been described elsewhere⁴. The autoradiographs were evaluated visually. Fractions or 'bands' of IgG or of viral IgG antibodies in the CSF which had no, or clearly less intensely stained counterparts, in matching serum were concluded to derive from intrathecal synthesis.

bodies to SV5 or any of the other viruses (Figs 1, 2) and did not provide evidence that the oligoclonal IgG bands reacted with SV5.

The seven patients with intrathecal SV5 antibody synthesis generally had a higher number of intrathecally synthesized virus antibody specificities than those without, and each had intrathecal synthesis of antibodies to at least two of three other (PF2, PF3, mumps) paramyxoviruses (Table 2). In each case, a close correspondence was seen between CSF patterns of SV5 antibodies and antibodies to PF2 (5 patients) or mumps (2 patients) (Figs 1 and 2), suggesting cross-reacting activities between these antibodies. This was confirmed by virus absorption⁷ of two selected CSF specimens, the first showing corresponding patterns of SV5 and PF2 antibodies and the second

of SV5 and mumps antibodies. Three separate 50 µl aliquots of concentrated CSF (IgG concentration 1 g l⁻¹) from each of the two patients were absorbed with 250 µl SV5, PF2, or mumps antigen preparations respectively, and analysed by IIF (data not shown). SV5 antibodies were removed after absorption with SV5 and PF2, but not with mumps antigen, in the first specimen, and after absorption with SV5 and mumps but not with PF2 in the second. The IIF patterns of other viral (measles, HSV) antibodies and of oligoclonal IgG bands in the absorbed specimens were essentially unchanged. The removal of oligoclonal IgG bands after absorption with SV5, reported by Goswami *et al.*³ in eight out of twelve patients, was therefore not confirmed here.

Using monoclonal antibodies, Randall *et al.* recently reported

TABLE 1 Viral IgG antibodies in serum and CSF and intrathecal antibody synthesis demonstrated by imprint immunofixation in 30 patients with MS

	Measles	HSV	VZV	Antibodies to		Mumps	PF2	PF3	SV5
				CMV					
Serum	29	25	30	22		25	22	26	19
CSF	29	25	30	22		25	22	26	19
Intrathecal synthesis	24	6	18	0		15	12	15	7

For methods see legend to Fig. 1.

TABLE 2 Oligoclonal IgG bands in the CSF and intrathecal virus antibody synthesis in 30 patients with MS, segregated according to presence or absence of intrathecal synthesis of antibodies to SV5

Intrathecal synthesis SV5 antibodies	CSF IgG bands	Intrathecal antibody synthesis								No. of different virus antibody specificities*				
		Measles	HSV	VZV	CMV	Mumps	PF2	PF3	Any virus	1	2	3	4	5
Yes: N=7	6	6	2	5	0	6	6	6	7				1	1
No: N=23	23	18	4	13	0	9	6	9	23	6	4	6	5	2
Total: N=30	29	24	6	18	0	15	12	15	30	6	4	7	6	7

For methods see legend to Fig. 1.

*SV5 antibodies excluded.

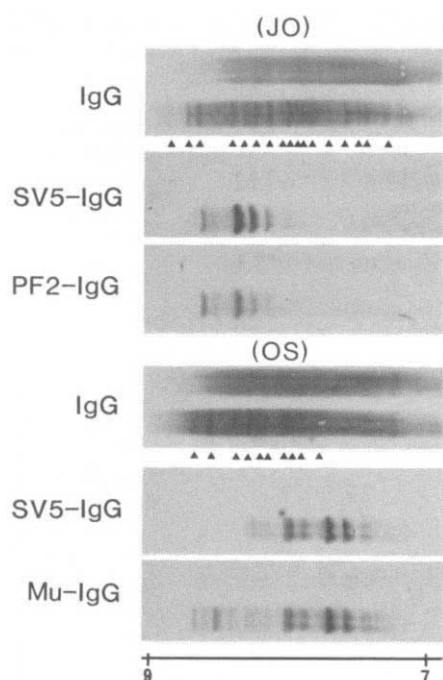


FIG. 2 Comparison of patterns of total IgG and viral antibodies in sera and CSF from two of the patients shown in Fig. 1. The specimens were electrofocused in a pH 6.5–9.5 gradient in order to provide better separation of IgG than in the pH 3.5–9.5 gradient shown in Fig. 1. Methods and symbols are otherwise as in Fig. 1. Note the difference in patterns between intrathecally synthesized SV5-IgG antibodies and bands of oligoclonal IgG (triangles) in both patients, and the correspondence between these antibodies and intrathecally synthesized antibodies to PF2 (case JO) or mumps (case OS).

the presence of cross-reacting epitopes on at least three virus structural proteins of SV5 and PF2, but not on the hemagglutinin-neuraminidase (HN) protein, which was unique to each virus⁸. Five of the monoclonal antibodies to SV5 HN used in this study (provided by Professor W. C. Russell) were analysed by IIF. Each reacted strongly with SV5 none reacted with PF2, showing that our method could readily detect antibodies to the SV5 HN protein. We conclude that methodological limitations do not explain the failure to detect antibodies reacting selectively with SV5 in the MS patients we studied.

In conclusion, our results suggest that there is no association between oligoclonal CSF IgG bands and SV5-specific antibodies in MS patients. The intrathecal synthesis of multiple viral antibodies constituting a minor fraction of the CSF IgG is generally thought to reflect a non-specific activation of B cells within the CNS, and is unlikely to be of aetiological importance. The oligoclonal IgG bands, however, represent a major fraction of the intrathecally synthesized immunoglobulin and may be recognizing major intrathecal immunogens involved in MS. Defining their specificity therefore remains a highly important challenge. □

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1. Laterre, E. C., Callewaert, A., Heremans, J. F., & Sfaello, Z. *Neurology* **20**, 982–990 (1970).
2. Johnson, K. P. *et al. Neurology* **27**, 273–277 (1977).
3. Goswami, K. K. A., Randall, R. E., Lange, L. S. & Russell, W. C. *Nature* **327**, 244–247 (1987).
4. Vartdal, F. & Vandvik, B. *J. Neurol. Sci.* **54**, 99–107 (1982).
5. Norrby, E. *et al. Infect. Immunity*, **10**, 688–694 (1974).
6. Salmi, A. A., Reunanen, M., Ilonen, J. & Panellius, M. *Clin. exp. Immun.* **52**, 241–249 (1983).
7. Vartdal, F., Vandvik, B. & Norrby, E. *Ann. Neurol.* **8**, 248–255 (1980).
8. Randall, R. E. & Young, D. F. *J. gen. Virol.* **69**, 2051–2060 (1988).

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Cloning of human telomeres by complementation in yeast

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TELOMERES confer stability on chromosomes by protecting them from degradation and recombination and by allowing complete replication of the end¹. They are genetically important as they define the ends of the linkage map. Telomeres of lower eukaryotes contain short repeats consisting of a G-rich and a C-rich strand, the G-rich strand running 5'–3' towards the telomere and extending at the end^{2–11}. Telomeres of human chromosomes share characteristics with those of lower eukaryotes^{12,13}, including sequence similarity as detected by cross-hybridization^{14–16}. Telomeric repeats from many organisms can provide telomere function in yeast^{2,17}. Here we describe a modified yeast artificial chromosome (YAC) vector with only one telomere which we used to clone human telomeres by complementation in yeast. YACs containing human telomeres were identified by hybridization to an oligonucleotide of the trypanosome telomeric repeat. A subcloned human fragment from one such YAC is immediately subtelomeric on at least one human chromosome.

Human DNA digested with restriction enzymes which have four base-pair (bp) recognition sequences displays a range of fragments which hybridize to oligonucleotides containing the trypanosome telomeric repeat sequence TTAGGG. In the case of *Sau3A*, this range of fragments is centred at about 10 kilobases (kb) in DNA isolated from peripheral blood cells. These fragments are lost after brief digestion of intact DNA with

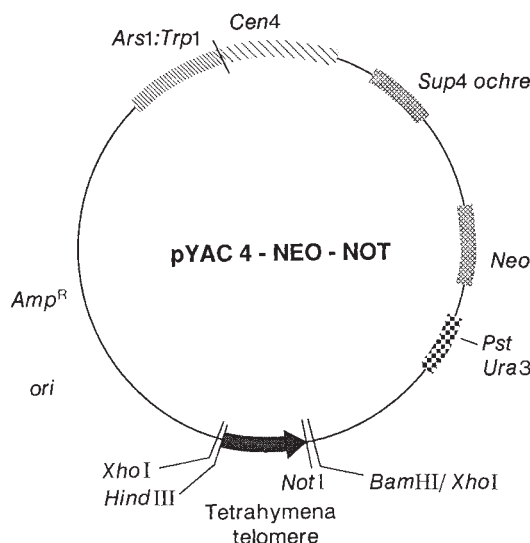


FIG. 1 Cloning of human sequences in pYAC4-Neo-Not. pYAC4-Neo¹⁶ was cut to completion with *Bam*HI and partially with *Xho*I. The adaptor sequence 5'-TCGAGGGATCCGCGCCGCGC3', base-paired with 15 of 19 bases, and having TCGA and GATC unpaired at the 5' ends, was ligated as described²⁸. The adaptor destroys the original *Bam*HI site and recreates the *Xho*I site. The *his3* gene and one of the tetrahymena telomeric repeats (TTGGGG)_n in pYAC4-Neo are deleted. A *Not*I restriction site is introduced adjacent to the remaining tetrahymena telomeric repeat and a *Bam*HI site between the *Not*I and *Xho*I sites. The structure shown was confirmed by restriction site mapping. Human placental DNA between 5 and 15 kb after *Sau*3A digestion was recovered from an agarose gel and ligated to pYAC4-Neo-Not which had been digested with *Not*I, dephosphorylated and digested with *Bam*HI. This DNA was used to transform AB1380 spheroplasts.

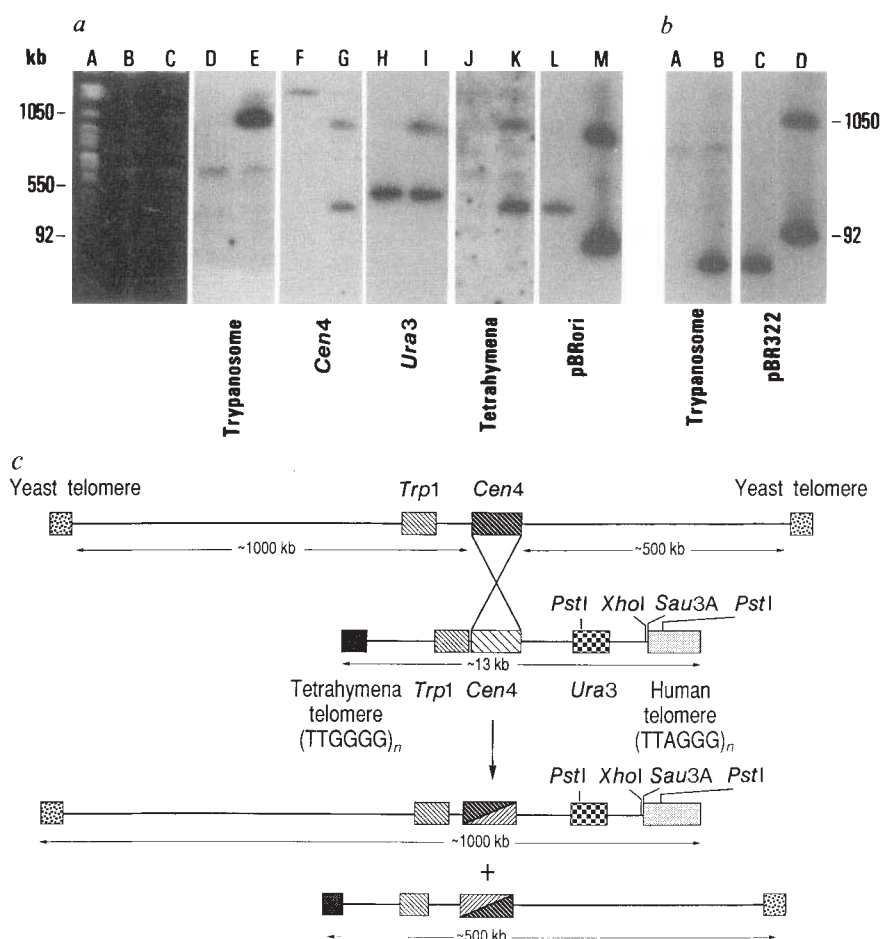
exonuclease *Bal31* (ref. 15). We have modified a YAC vector pYAC4 Neo (ref. 18) to give a vector (pYAC4-Neo-Not) which requires the addition of a second sequence with telomere function for its maintenance as a linear minichromosome in yeast. The presence of the *sup4* gene in the vector provides a colour test for the maintenance of this marker; AB1380 (ref. 19) yeast colonies are red in the absence and white in the presence of this gene. Details of the vector are given in the legend to Fig. 1. *Sau3A*-digested placental DNA from 5–15 kb was used as an enriched source of human telomeres for ligation to pYAC4-Neo-Not digested with *Not1* and *BamHI*.

AB1380 was transformed²⁰ with the ligation mix and selected for *Ura*⁺ transformants. *Ura*⁺ transformants (240) were screened for the presence of human telomeric sequences on the basis of colony hybridization to the oligonucleotide (TTAGGG)₄. Two strongly hybridizing positive colonies were found (Hutel 1 and Hutel 22). Pulsed-field gel electrophoresis (PFG) comparison of AB1380 and Hutel 1 chromosomes showed that in this recombinant the 1.5 megabase (Mb) yeast chromosome 4 was replaced by two new chromosomes of 1.0 and 0.5 Mb respectively (Fig. 2a). Hybridization analysis shows that these chromosomes are the product of recombination between the transforming molecule and yeast chromosome 4 (Fig. 2a, c). In the case of Hutel 22, PFG analysis showed the presence of a 12-kb linear artificial chromosome, as shown in Fig. 2b. When plated on non-selective media, a high proportion of colonies were red as we expected, because a linear chromosome of this size should be relatively unstable²¹. In contrast, Hutel 1 gave less than 5 per cent red colonies when plated on non-selective media (data not shown).

We wished to know if the human sequences in Hutel 1 and Hutel 22 were present at the end of the new yeast chromosomes. First, the end of a DNA molecule presents as an apparent site for all restriction enzymes. Such a site was present at the distal end of the region of human DNA homologous to the (TTAGGG)₄ oligonucleotide probe for both Hutel 1 and Hutel 22 (data not shown). Two additional features should be exhibited by terminal restriction fragments with human homology. They should be decreased in size and eventually become undetectable when intact recombinant yeast DNA is digested with exonuclease *Bal31*. Second, these terminal fragments should be heterogeneous in size when compared with fragments of the genome which are bounded by two restriction sites²². Figure 3 shows that both of these criteria are satisfied for Hutel 1. We consider that the most likely explanation for this heterogeneity is the addition of yeast terminal repeats. We conclude that Hutel 1 and Hutel 22 contain human DNA fragments with homology to trypanosome telomeric repeats and that these sequences support telomere function in a yeast.

Although >95 per cent of sequences which hybridize to (TTAGGG)₄ are *Bal31*-sensitive in intact human DNA and hence presumably telomeric or closely subtelomeric, it was important to show that the human sequences that we cloned in yeast are telomeric in the human genome. We have cloned some of the human sequences from Hutel 1 into a plasmid (pHutel 1), as described in the legend to Fig. 4. This cloned DNA fragment has no homology to the (TTAGGG)_n terminal repeats detectable by hybridization or by sequence analysis (data not shown). When hybridized to blots of human DNA treated with *Bal31* for different times and then digested with *Sau3A*, this

FIG. 2 Novel yeast chromosomes with human telomeres. *a*, Hutel 1 and AB1380 analysed by pulsed field gel electrophoresis. A–C, ethidium bromide stained gel; D and E, probed with (TTAGGG)₄; F and G, probed with a yeast *cen4* DNA fragment; H and I, probed with yeast *ura3*; J and K, probed with (TTGGGG)₄; L and M, probed with pBR *ori* probe (*PvuII*–*PstI*). A and M, yeast marker chromosomes; B, D, F, H and J, AB1380; C, E, G, I, K and L, Hutel 1. The larger of the novel chromosomes hybridized to (TTAGGG)₄ and to *ura3*. Both novel chromosomes hybridized to a *cen4* probe and the smaller chromosome hybridized with the *ori* region of pBR322 and the tetrahymena telomere oligonucleotide probe (TTGGGG)₄. Restriction analysis of Hutel 1 DNA showed that endogenous fragments were retained unchanged in size for the *trp1* and *ura3* genes, but that vector and endogenous *cen4* fragments were changed in size (data not shown). Homologous recombination between the *cen4* region of the vector and the original chromosome 4 would give rise to these chromosomes as detailed (Fig. 2c). *b*, Hutel 22 and AB1380 analysed by pulsed field gel electrophoresis. A and B probed with (TTAGGG)₄; C and D probed with pBR322. A, AB1380; B and C, Hutel 22; D, YP148 marker chromosomes. Yeast DNAs were prepared in agarose plugs as described²⁹. They were run on a 1% agarose gel overnight using a waltzer apparatus. The gel was made and run in 0.25 × TBE (0.089 M Tris, 0.089 M boric acid, 0.002 M EDTA). Conditions were 7 V cm⁻¹ at 10 °C and 90° between 120° reorientations. The gel was blotted to Hybond-N and hybridized with the ³²P-labelled probes as listed above. Hybridization with random primed fragments was overnight at 68 °C in 7% SDS, 0.5 M Na₂HPO₄, pH 7.2, 0.5% dried milk powder. Filters were washed in 0.1 × SSC, 0.1% SDS at 65 °C. Hybridization with oligonucleotides was overnight at 50 °C in 0.1% SDS, 0.1% sodium pyrophosphate, 0.05% bovine serum albumin, 0.05% polyvinylpyrrolidone, 0.05% Ficoll, 5 × SSC. Filters were washed in 4 × SSC, 0.1% SDS at 65 °C. Size markers (kb) are indicated. *c*, Recombination between



endogenous and artificial chromosomes. Restriction sites relevant to the chromosome construction and subcloning are indicated. Figure is not to scale.

probe detected *Bal*31-sensitive fragments which were the same size as those detected by a (TTAGGG)₄ oligonucleotide probe (Fig. 4a). In addition, a number of discrete fragments are detected which do not correspond in size to fragments detected by the (TTAGGG)₄ probe. When hybridized to digests of blood and sperm DNA from different individuals, pHutel 1 DNA detected heterogeneous smears which were the same size as those detected by the oligonucleotide (TTAGGG)₄ and which were larger in sperm than in blood DNAs (Fig. 4b). This size

difference between these tissues from a single individual is a characteristic of the human pseudoautosomal telomeres¹³. From the restriction map presented in Fig. 2, we would predict that this probe would not detect heterogeneous fragments in a *Pst*I digest of human DNA. As shown in Fig. 4b, only discrete bands are detected in *Pst*I digests by pHutel 1. We conclude that this sequence must be immediately subtelomeric in the human genome.

Telomeres of all organisms studied consist of short tandemly

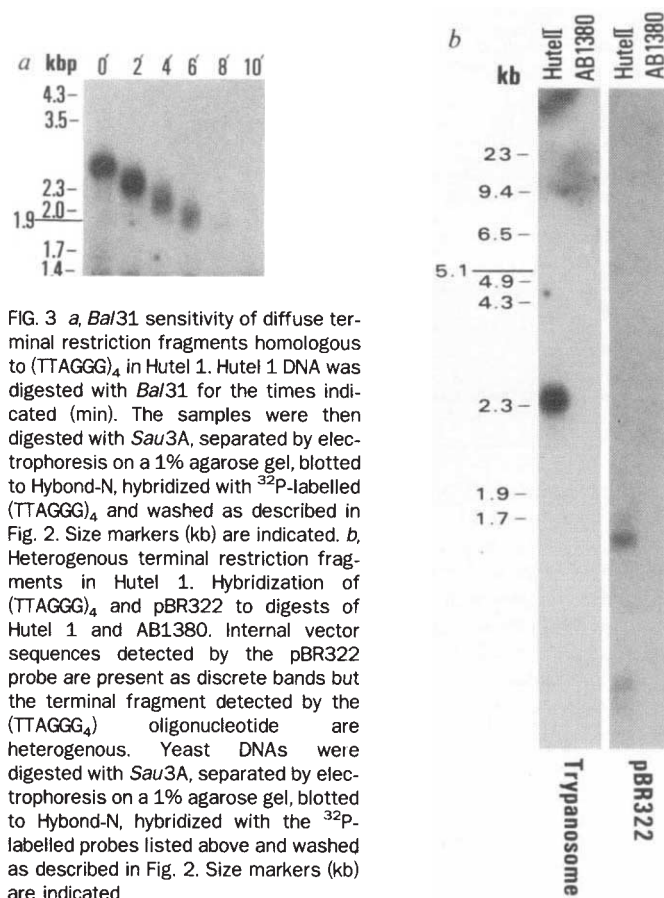


FIG. 3 a, *Bal*31 sensitivity of diffuse terminal restriction fragments homologous to (TTAGGG)₄ in Hutel 1. Hutel 1 DNA was digested with *Bal*31 for the times indicated (min). The samples were then digested with *Sau*3A, separated by electrophoresis on a 1% agarose gel, blotted to Hybond-N, hybridized with ³²P-labelled (TTAGGG)₄ and washed as described in Fig. 2. Size markers (kb) are indicated. b, Heterogenous terminal restriction fragments in Hutel 1. Hybridization of (TTAGGG)₄ and pBR322 to digests of Hutel 1 and AB1380. Internal vector sequences detected by the pBR322 probe are present as discrete bands but the terminal fragment detected by the (TTAGGG)₄ oligonucleotide are heterogeneous. Yeast DNAs were digested with *Sau*3A, separated by electrophoresis on a 1% agarose gel, blotted to Hybond-N, hybridized with the ³²P-labelled probes listed above and washed as described in Fig. 2. Size markers (kb) are indicated.

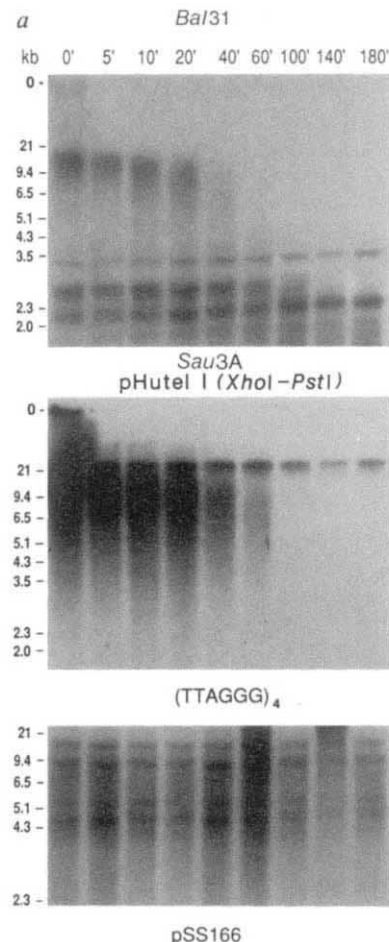
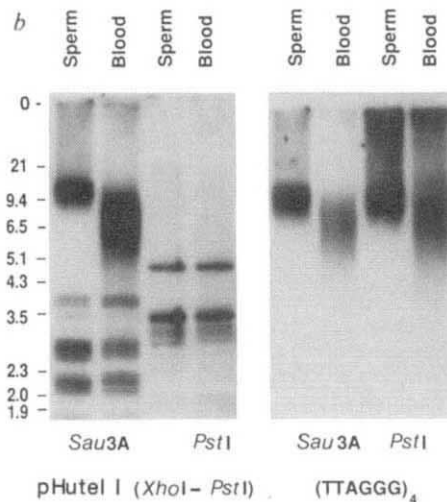


FIG. 4 Terminal location of pHutel 1 human sequences in the human genome. The human sequence in Hutel 1 was restriction mapped and a *Pst*I site proximal to the terminal repeats was found which, in the recombinant, lies on a *Pst*I fragment which also contains part of the yeast marker *ura3*. This fragment (pHutel 1) was recovered from a *Pst*I library of Hutel 1 in pTZ 19 using a *ura3* DNA fragment as a probe. a, Human placental DNA was digested with *Bal*31 for the times indicated (min). The samples were then split and digested with *Sau*3A or *Pst*I, separated by electrophoresis on 0.8% agarose gels and blotted to Hybond-N. The filter with the *Bal*31 series digested with *Sau*3A was first hybridized with ³²P-labelled XhoI-PstI fragment containing human sequences from pHutel 1 and washed as described in Fig. 2. The filter was then stripped as recommended by the manufacturers, hybridized with ³²P-labelled (TTAGGG)₄ and washed as described in Fig. 2. The filter with the *Bal*31 series digested with *Pst*I was hybridized with ³²P-labelled pSS166 (*Hind*III fragment from cosmid B4 (ref. 30)). Hybridization was at 60 °C, but otherwise as described in Fig. 2. The filter was washed in 2 × SSC, 0.1% SDS at 65 °C. ○, Gel origin; marker sizes are given in kb. b, Blood and sperm DNAs from the same individual were digested with *Sau*3A or *Pst*I, separated by electrophoresis on a 0.8% agarose gel, blotted to Hybond-N and hybridized with the ³²P-labelled probes as indicated. Hybridization and washing conditions were as described in Fig. 1. Size markers (kb) are indicated; ○, gel origin.



repeated sequences. Variation in copy number of these repeats results in the heterogeneity observed for terminal restriction fragments^{23,24}. In yeast the terminal repeats are the only elements required for telomere function²², and telomeric repeats from a number of organisms are capable of function in yeast by the addition of yeast sequences. Previous data¹²⁻¹⁵ suggested that human telomeres showed many of the structural features of telomeres of lower eukaryotes. We have shown here that a telomeric fragment of human DNA can function as a telomere in yeast, suggesting that the structure of the ends of human chromosomes conforms to the general model of a number of short tandem repeats with a G-rich strand and a C-rich strand: the G-rich strand runs 5'-3' towards the end of the molecule and ends in a short single-strand extension. This degree of conservation and cross-kingdom function may reflect some similarities of replication mechanisms and protein interaction at the chromatin level. In other organisms there are proteins which interact specifically with the telomeres^{24,25}. Alternatively, these sequences may themselves form functionally significant structures. G-G base pairing has been detected in tetrahymena telomeric sequences²⁶ and there may also be a requirement for the ability to form a four-stranded parallel base-paired structure in meiosis²⁷.

Cloning of human telomeres in yeast provides a powerful method of obtaining markers at the ends of the physical and genetic maps of the human genome. Linkage of these markers to distal markers on the human linkage map will define the length of this map. □

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1. Blackburn, E. H. & Szostak, J. W. *A. Rev. Biochem.* **53**, 163-194 (1984).
2. Shampay, J., Szostak, J. W. & Blackburn, E. H. *Nature* **310**, 154-157 (1984).
3. Walmsley, R. W., Chan, C. S. M., Tye, B.-K. & Petes, T. D. *Nature* **310**, 157-160 (1984).
4. Sugawara, N. & Szostak, J. W. *Yeast* **2** (Suppl.) 373 (1986).
5. Blackburn, E. H. & Gall, J. G. *J. molec. Biol.* **120**, 33-53 (1978).
6. Klobutcher, L. A., Swanton, M. A., Donini, P. & Prescott, D. M. *Proc. natn. Acad. Sci. U.S.A.* **78**, 3015-3019 (1981).
7. Ponzi, M., Pace, T., Dore, E. & Frontali, C. *EMBO J.* **4**, 2991-2995 (1985).
8. Emery, H. S. & Weiner, A. M. *Cell* **26**, 411-419 (1981).
9. Blackburn, E. H. & Challoner, P. B. *Cell* **36**, 447-457 (1984).
10. Van der Ploeg, L. H. T., Liv, A. Y. C. & Borst, P. *Cell* **36**, 459-468 (1984).
11. Forney, J., Henderson, E. R. & Blackburn, E. H. *Nucleic Acids Res.* **15**, 9143-9152 (1987).
12. Cooke, H. J., Brown, W. R. A. & Rappold, G. A. *Nature* **317**, 687-692 (1985).
13. Cooke, H. J. & Smith, B. A. *Cold Spring Harbor Symp. quant. Biol.* **51**, 213-219 (1986).
14. Allshire, R. C. *et al. Nature* **332**, 656-659 (1988).
15. Moyzis, R. K. *et al. Proc. natn. Acad. Sci. U.S.A.* **85**, 6622-6626 (1988).
16. Richards, E. J. & Ausubel, F. M. *Cell* **53**, 127-136 (1988).
17. Pluta, A. F., Dani, G. M., Spear, B. B. & Zakian, V. A. *Proc. natn. Acad. Sci. U.S.A.* **81**, 1475-1479 (1984).
18. Cooke, H. J. & Cross, S. H. *Nucleic Acids Res.* **16**, 11817 (1988).
19. Burke, D. T., Carle, G. F. & Olsen, M. V. *Science* **236**, 806-812 (1987).
20. Burgers, P. M. J. & Percivals, K. *J. analyt. Biochem.* **163**, 391-397 (1987).
21. Murray, A. W., Schultes, N. P. & Szostak, J. W. *Cell* **45**, 529-536 (1986).
22. Szostak, J. W. & Blackburn, E. H. *Cell* **29**, 245-255 (1982).
24. Gottschling, D. E. & Zakian, V. A. *Cell* **47**, 195-205 (1986).
25. Berman, J., Tachibana, C. Y. & Tye, B.-K. *Proc. natn. Acad. Sci. U.S.A.* **83**, 3713-3717 (1986).
27. Sen, D. & Gilbert, W. *Nature* **334**, 364-366 (1988).
28. Lathe, R., Kierny, M. P., Skory, S. & Lecocq, J. P. *DNA* **3**, 173-182 (1984).
29. Bellis, M., Pages, M. & Roizes, G. *Nucleic Acids Res.* **15**, 6747 (1987).
30. Nakaseko, Y., Adachi, Y., Funahashi, S., Niwa, O. & Yanagida, M. *EMBO J.* **5**, 1011-1021 (1986).

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Molecular cloning of human telomeres in yeast

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TELOMERES are the DNA sequences found at the ends of linear chromosomes. They define the boundaries of the genetical and physical maps of such chromosomes and so are particularly important for the complete mapping of large genomes that is now being attempted. Telomeres have been intensively studied in the yeast *Saccharomyces cerevisiae* and in ciliated protozoa¹: in these organisms the telomeric DNA consists of arrays of tandemly repeated short sequences in which one strand is guanosine-rich and oriented 5' to 3' towards the chromosome end. The conservation of these structural features is reflected in the observation that telomeric DNA from a variety of protozoa will function as telomeres on artificial linear mini-chromosomes in yeast^{1,2}. Tandem arrays of the sequence TTAGGG have been identified at the telomeres of humans and other mammals³ and also of trypanosomes⁴. This indicates that the structural features of telomeres are conserved between higher and lower eukaryotes and implies that human telomeric DNA could function in yeast. I have used this idea to develop a strategy to isolate a specific human telomere as a molecular clone in yeast and have devised a simple and effective way of cloning other human telomeres and their associated sequences.

Human telomeres contain the sequence (TTAGGG)_n. This sequence is identical to the most prominent components of both the α -satellite DNA of guinea pig⁵ and the HS- α -satellite of DNA of kangaroo rat⁶. These satellite DNA sequences can be purified from the bulk of the genomic DNA by isopycnic centrifugation on a Cs₂SO₄ gradient in the presence of silver ions, so human telomeric DNA could possibly be similarly purified. Accordingly, I restricted human placental DNA with *Bam*HI and fractionated it on such a gradient. Hybridization analysis (Fig. 1a) using the oligonucleotide probe (TTAGGG)₄ revealed

that the telomeric DNA had been separated from the bulk of the genomic DNA. Fractions enriched in the sequence (TTAGGG)_n were pooled and their enrichment with respect to the unfractionated DNA was calculated after gel electrophoresis, filter transfer and hybridization analysis (Fig. 1b). Densitometry indicated that these sequences had been enriched sevenfold with respect to the bulk of the genomic DNA.

The pseudoautosomal region of the human sex chromosomes includes a telomere. Sequences originating about 25 kilobase pairs (kb) from this telomere have been isolated as molecular clones in bacteria⁷. One of the sequences, 29cl, lies on the telomeric *Bam*HI fragment. I used this *Bam*HI fragment as a model to test whether a human telomere could function in the yeast *S. cerevisiae* by constructing a vector, pTV2 (Fig. 2a), for cloning telomeres in yeast. Digestion of this molecule with *Bam*HI and *Not*I generates two fragments: at one end of the larger is a sequence (denoted in Fig. 2a by 'TEL') that is derived from the ribosomal DNA of *Tetrahymena thermophila*⁸ and which can function as a telomere in yeast, and at the other is a *Bam*HI site which could act as a cloning site. This molecule possesses only a single telomere and thus cannot autonomously function as a linear molecule in yeast cells. But if the telomere from the pseudoautosomal region can function as a telomere in yeast, then ligation of the *Bam*HI fragment (defined by 29cl) to the appropriately cut pTV2 will generate a molecule capable of autonomous linear existence in yeast. Accordingly, the telomere-enriched, *Bam*HI-cut DNA from the Ag⁺/Cs₂SO₄ gradient was ligated to vector DNA cut with *Not*I and *Bam*HI and introduced into yeast. This generated ~2,600 transformants. (An undefined proportion of these transformants contain circular molecules derived from the large *Bam*HI/*Not*I fragment of pTV2 by illicit intramolecular ligation within the yeast, hence the complexity of the potential telomere library is as yet unknown.) The transformants were then divided into ten pools which were individually amplified and screened for the 29cl sequence by colony hybridization. Two pools each originating from the same transformation plate contained hybridizing colonies. These were isolated and examined for the presence of a linear mini-chromosome of the appropriate structure by digestion with restriction enzymes and pulsed-field gel electro-

FIG. 1 Enrichment of human telomeric DNA by isopycnic ultracentrifugation on $\text{Ag}^+/\text{Cs}_2\text{SO}_4$ gradient. *a*, Spectrophotometric and hybridization analysis of fractions from the gradient. *b*, Hybridization analysis of DNA enriched for telomeric sequences. METHODS. *a*, A solution of *Bam*HI-digested placental DNA (1.2 ml) at 1.0 mg ml⁻¹ in 0.1 M Na₂SO₄, 0.1 M borate buffer, pH 9.2, was slowly mixed with 16.2 mM AgClO₄ to give a silver:phosphate molar ratio of 0.2. This was added to a solution of Cs₂SO₄ (density 1.566 g ml⁻¹) in 0.1 M Na₂SO₄ to give a final volume of 24.7 ml, a borate concentration of 0.05 M and a density of 1.5 g ml⁻¹. The mixture was centrifuged at 50,000 r.p.m. for 50 h in a Beckman VTi rotor. After centrifugation, the tube was pierced and 0.24 ml fractions collected from the bottom. Fractions were monitored spectrophotometrically and by dot-blot filter hybridization using a (TTAGGG)₄ probe. The hybridization and washing were carried out as described⁹ at 50 °C. The (TTAGGG)_n sequence detected by this probe banded between 1.55 and 1.53 g ml⁻¹, which is consistent with the results obtained for guinea pig α -satellite DNA¹⁰. *b*, 5 μ g human male *Bam*HI-digested placental DNA or 0.7 μ g telomere-enriched, *Bam*HI-digested DNA were electrophoresed on an 18 cm, 0.6% agarose gel for 16 h at 1.5 V cm⁻¹. The gel was stained with ethidium bromide and photographed under ultraviolet light (left panel) and then filter-transferred and analysed with the (TTAGGG)₄ probe as described above (right panel).

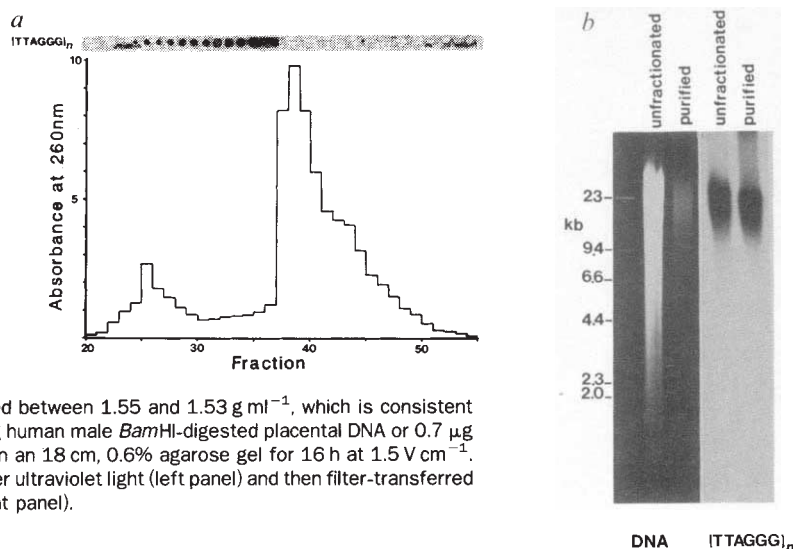
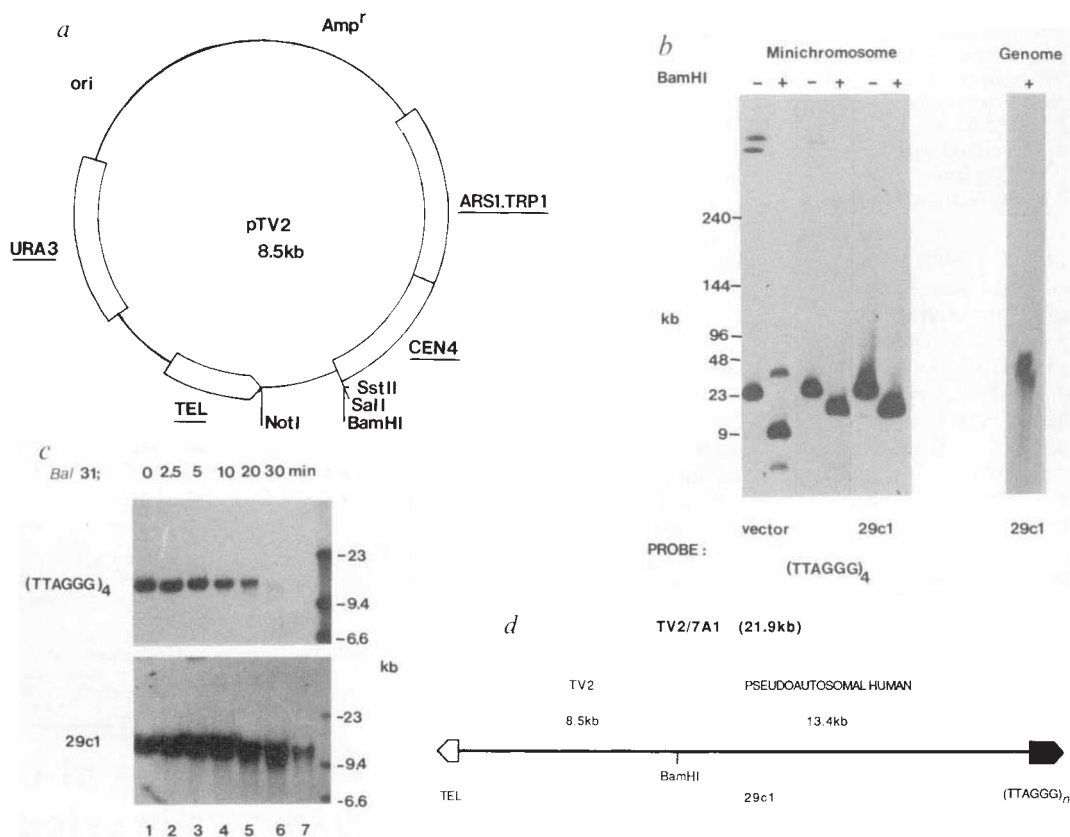


FIG. 2 Molecular cloning of the telomere from the human pseudoautosomal region and mapping of a minichromosome containing this telomere. *a*, Structure of pTV2. *b*, Restriction site analysis of the minichromosome TV2/7A1 containing the human pseudoautosomal telomere. *c*, Digestion with *Bal*31 and *Bam*HI reveal the relative orientation of 29cl and (TTAGGG)_n in TV2/7A1. *d*, Molecular map of TV2/7A1.

METHODS. *a*, pTV2 was constructed in *E. coli* from pYAC4¹¹, a polylinker containing a *Not*I site and a polylinker containing *Bam*HI, *Sal*I and *Sst*II sites, using standard techniques. The vector and construction details are available on request. *b*, Telomere-enriched DNA (67 μ g) was prepared as described in the legend to Fig. 1 and ligated to 25 μ g dephosphorylated *Bam*HI-, *Not*I-cut pTV2 in 300 μ l. Unligated vector was removed from the ligation mix by preparative gel electrophoresis in low-gelling-temperature agarose (Seakem). Agarose containing the target DNA that was ligated to vector was melted and digested with *Agarase* (Calbiochem). The digest was extracted with phenol, then with chloroform and dialysed against 0.01 M Tris.HCl, 0.001 M EDTA, pH 7.4, and concentrated to 300 μ l by vacuum dialysis. The ligated DNA was then introduced into *S. cerevisiae* strain AB1380¹¹ by spheroplast transformation¹². The transformants were selected on medium lacking uracil and collected in ten pools of ~200 clones, then ~10,000 cells from each pool were plated on to 20 cm-square Hybond-N membranes and screened by colony hybridization^{13,14} using the 29cl probe. Single colonies containing cognate DNA were then grown and used to prepare DNA in agarose plugs¹⁵. DNA from one clone that contained the minichromosome TV2/7A1 was analysed by pulsed-field gel electrophoresis¹⁶ and filter-hybridization, either with (+) or without (-) prior *Bam*HI digestion. For comparative purposes, the *Bam*HI-digested male placental DNA (10 μ g) was run on the same gel. The probes used in the hybridization analysis were either the large *Xho*I-*Xho*I fragment of pYAC4 (vector), the oligonucleotide (TTAGGG)₄, or 29cl. The 29cl and the vector probes were used as described previously¹⁷. *c*, Agarose plugs containing ~1 μ g yeast genomic DNA and the mini-chromosome TV2/7A1 in 0.04 ml were digested at 30 °C for the time indicated with 0.5 units of *Bal*31 (BRL) in 0.07 ml. The digest



was stopped by adding 1 ml 0.02 M EGTA, 0.01 M Tris.HCl, 0.001 M EDTA, pH 7.4. After 20 min, the plug was rinsed in water, gelatin, salts and dithiothreitol as appropriate, and the DNA digested to completion with *Bam*HI. Before loading on to a 0.8% agarose gel the plugs were rinsed in 10 mM Tris.HCl, 1 mM EDTA, pH 7.4, and melted. The gels were ethidium bromide-stained and photographed under ultraviolet light to reveal the absence of any detectable endonuclease activity in the *Bal*31. They were then filter-transferred and analysed with the probes indicated, as described above. No DNA was loaded into track 7 of either gel, but in the gel shown in the lower panel, molten agarose leaked from track 6 into track 7 to give rise to hybridizing material in this track. Track 8 of each panel includes end-labelled *Hind*III fragments of phage λ DNA as size markers. *d*, When an exogenous telomere is introduced into yeast, it acts as the substrate for a terminal transferase that adds telomeric repeats which are characteristic of yeast to the pre-existing G-rich repeat^{18,19}. The novel array characteristic of yeast typically extends for only a few hundred base pairs and is not illustrated on this map.

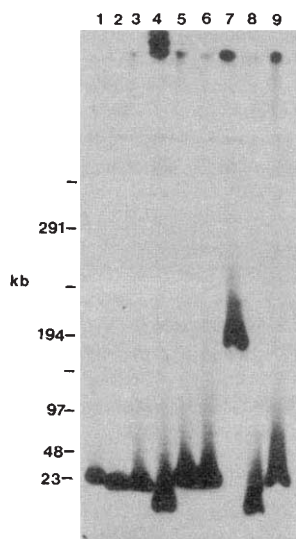


FIG. 3 Mini-chromosomes containing candidate human telomeres. The amplified population of transformants described in the legend to Fig. 2b was screened with the oligonucleotide (TTAGGG)₄; clones that hybridized to this probe, but not to the 29cl sequence, were picked, cultured and used to prepare high-molecular weight DNA. These DNA samples were then analysed (tracks 2-9), together with that from the clone containing TV2/7A1 (track 1), by pulsed-field gel electrophoresis and filter-hybridization with the (TTAGGG)₄ probe. The two members of each pair of clones that migrate with identical mobility originate from distinct cloning events.

phoresis. They all contained a 22-kb molecule which is absent from the host strain and which hybridized to 29cl. The structure of the molecule, TV2/7A1, in cells derived from one colony was examined in more detail (Fig. 2b). TV2/7A1 hybridized to vector DNA, to 29cl and to the oligonucleotide (TTAGGG)₄ (Fig. 2b). *Bam*HI cleaved the molecule into two fragments whose sizes summed to that of the uncut minichromosome. One fragment of ~9 kb contained vector sequences (Fig. 2b), the other fragment of 13.4 kb contained the 29cl and (TTAGGG)₄ sequences (Fig. 2b). (In addition to the pTV2-derived fragment there are fragments in the *Bam*HI digest which hybridize to the vector; these result from hybridization to host genomic fragments.) If the (TTAGGG)_n sequence on the pseudoautosomal fragment in the mini-chromosome has been recognized as a telomere by the yeast, then it should be susceptible to the action of the exonuclease *Bal*31. The 29cl sequence, on the other hand, should lie in the middle of the molecule and be relatively resistant to the action of this exonuclease. These predictions were tested by digesting DNA containing the mini-chromosome for progressively increasing lengths of time with *Bal*31, cutting with *Bam*HI, followed by Southern blotting and filter hybridization using either the (TTAGGG)₄ probe (Fig. 2c, upper panel) or the 29cl probe (Fig. 2c, lower panel). These results are summarized in the map in Fig. 2d. Sizing of the fragments produced by the action of *Bal*31 and *Bam*HI indicates that the (TTAGGG)_n array extends for only 1.2 kb at the end of this mini-chromosome. But in human placental DNA, the sizes of the arrays with this sequence range from 9 kb to 20 kb (unpublished observations, and ref. 3). It thus seems likely that the (TTAGGG)_n sequence is being abbreviated during the cloning procedure. This could be a consequence of the way in which the human telomere is healed in the yeast, although some exogenous tandemly repeated sequences are unstable in yeast artificial chromosome cloning vectors in a way that is reminiscent of their instability in *Escherichia coli* plasmid and phage vectors (C. Tyler-Smith and A. Villasante, personal communication). It is possible that such instability is contributing to the loss of these sequences from the chimaeric mini-chromosome. The observation (Fig. 2b) that the pseudoautosomal probe 29cl recognized a 30 kb fragment

in the *Bam*HI-cut placental DNA used for cloning is consistent with this explanation, although little is known about the structure of human proterminal DNA and more complex rearrangements could have occurred in the course of the cloning experiment. Further mapping experiments will resolve this point.

The observation that the pseudoautosomal telomere can function as a telomere in yeast and contains the sequence (TTAGGG)_n indicates that other transformants in the pTV2 *Bam*HI-fragment library with this sequence could contain linear minichromosomes. I investigated this prediction by screening the amplified library with a (TTAGGG)₄ probe. Eight additional independent clones identified in this way were colony-purified and analysed by pulsed-field gel electrophoresis (Fig. 3). Each clone contains a minichromosome which includes (TTAGGG)_n. The electrophoretic behaviour of these molecules indicated that they were probably linear and were between 10 and 200 kb long. Although there may be blocks of the (TTAGGG)_n array elsewhere in the genome, it is reasonable to regard these molecules as candidates for novel unmapped human telomeres.

These results demonstrate a simple and novel strategy for isolating human telomeres and their flanking sequences. This will allow analysis of the structure and function of human proterminal DNA and will define the limits of the human genome map. In light of the evolutionary conservation of the distinctive structural and functional characteristics of telomeric DNA, the approaches described here could be applicable to a wide variety of organisms. □

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1. Blackburn, E. H. & Szostak, J. W. *A. Rev. Biochem.* **53**, 163-194 (1984).
2. Szostak, J. W. & Blackburn, E. H. *Cell* **29**, 245-255 (1982).
3. Moyzis, R. K. et al. *Proc. natn. Acad. Sci. U.S.A.* **85**, 6622-6626 (1988).
4. Blackburn, E. H. & Challoner, P. B. *Cell* **38**, 447-457 (1984).
5. Southern, E. M. *Nature* **227**, 794-798 (1970).
6. Fry, K. & Salsler, W. *Cell* **12**, 1069-1084 (1977).
7. Cooke, H. J., Brown, W. R. A. & Rappold, G. A. *Nature* **317**, 687-692 (1985).
8. Blackburn, E. H. & Gall, J. G. *J. Molec. Biol.* **120**, 33-53 (1978).
9. Wallace, R. B. & Thein, S. L. in *Human Genetic Diseases* (ed. Davies, K. E.) (IRL, Oxford, 1986).
10. Corneo, G., Ginelli, E., Soave, E. & Bernardi, G. *Biochemistry* **7**, 4373-4379 (1968).
11. Burke, D. T., Carle, G. & Olson, M. *Science* **236**, 806-812 (1987).
12. Burgers, P. M. J. & Percival, K. J. *Analyt. Biochem.* **163**, 391-397 (1987).
13. Coulson, A., Waterston, R., Kiff, J., Sulston, J. & Kohara, Y. *Nature* **335**, 184-186 (1988).
14. Sherman, F., Fink, G. R. & Hicks, J. B. *Methods in Yeast Genetics* (Cold Spring Harbor, New York, 1986).
15. Schwartz, D. C. & Cantor, C. *Cell* **37**, 67-75 (1984).
16. Southern, E. M., Anand, R., Brown, W. R. A. & Fletcher, D. S. *Nucleic Acids Res.* **15**, 5925-5943 (1987).
17. Brown, W. R. A. *EMBO J.* **7**, 2377-2385 (1988).
18. Shampay, J., Szostak, J. W. & Blackburn, E. H. *Nature* **310**, 154-157 (1984).
19. Grieder, C. W. & Blackburn, E. H. *Cell* **51**, 887-898 (1987).

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Identification of the electron transfers in cytochrome oxidase that are coupled to proton-pumping

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MITOCHONDRIAL cytochrome oxidase is a functionally complex, membrane-bound respiratory enzyme which catalyses both the reduction of O₂ to water and proton-pumping. During respiration, an exogenous donor, cytochrome *c*, donates four electrons to O₂ bound at the bimetallic haem a₃ Fe-Cu centre within the enzyme. These four electron transfers are mediated by the enzyme's haem *a* and Cu_A redox centres and result in the translocation of four

protons across the inner mitochondrial membrane.¹ The molecular mechanism of proton translocation has not yet been delineated, however, and in the absence of direct experimental evidence all four electron transfers have been assumed to couple equally to proton-pumping. Here, I report the effects of proton-motive force and membrane potential on two equilibria involving intermediates of the bimetallic centre at different levels of O₂ reduction.²⁻⁷ The results show that only two of the electron transfers, to the 'peroxy' and 'oxyferryl' intermediates of the bimetallic centre, are linked to proton translocation, a finding which strongly constrains candidate mechanisms for proton-pumping.

The assumption that all four electron transfers from cytochrome *c* to O₂ bound to the bimetallic centre are equally coupled to proton-pumping (Fig. 1a) was tested by determining the linkage of two of the one-electron transfer reactions (Fig. 1b, box I) to proton and charge translocation. This was made possible by the observation that the catalytic cycle of the bimetallic centre can be driven in reverse, with discrete oxidation of intermediate O to F, and further to P, and concomitant reversed electron transfer to cytochrome *c*⁵⁻⁷. In isolated mitochondria, intermediates O, F and P can be brought in near equilibrium with cytochrome *c* (redox-poised with ferri-/ferrocyanide) by a proton-motive force across the membrane. Thus, the dependence of the P/F and F/O redox equilibria on proton-motive force and membrane potential ($\Delta\psi$), at constant pH and redox potential of cytochrome *c*, provided information on the coupling of each electron transfer reaction to proton and charge translocation.

Figure 2 shows the dependence of the F/O and P/F redox equilibria on phosphorylation potential. Proton-motive force is generated by ATP hydrolysis which is catalysed by the mitochondrial H⁺-ATPase. The data indicate that electron transfer from cytochrome *c* to P, and to F, is coupled to the synthesis of 0.9–1.0 and 0.7–0.75 ATP molecules, respectively. This means that 1.6–1.75 ATP molecules are formed by oxidative phosphorylation coupled to the transfer of two electrons from cytochrome *c* to reduce P to O (through F). This 'local' ATP/2e⁻ ratio is nearly twice that of overall cytochrome *c* oxidase activity⁸. It follows that the electron transfers from cytochrome *c* to P and to F constitute the main energy-conservation steps in the catalytic cycle of cytochrome oxidase. By contrast, the two remaining electron transfers, from cytochrome *c* to O and to H (Fig. 1b) make only a small contribution to ATP synthesis, which may be limited to the electrogenicity of electron transfer from cytochrome *c* to the bimetallic centre (Fig. 1a).

A quantitative evaluation of the linkage of the P/F and F/O reactions to proton and charge translocation requires a knowledge of the number of protons translocated per hydrolysed extramitochondrial ATP. At present an H⁺/ATP ratio of 4 is widely accepted⁹. From Fig. 2 it therefore follows that the P/F and F/O transitions are coupled to the overall translocation of 3.6–4.0 and 2.8–3.0 H⁺, respectively. It should be noted, however, that two events other than proton-pumping are electrogenic in the oxidase reaction (Fig. 1a). The first is electron transfer between cytochrome *c* and the binuclear centre; the

second is the uptake of the protons from the inside of the inner mitochondrial membrane that are consumed in reduction of O₂ to water¹⁰.

Table 1 summarizes the quantitative analysis of the results shown in Fig. 2, from which it can be concluded that both the P/F and the F/O transition are coupled to the pumping of close to 2 H⁺.

Figure 3 shows the dependence of the F/O equilibrium on electrical membrane potential at high pH, where the intermedi-

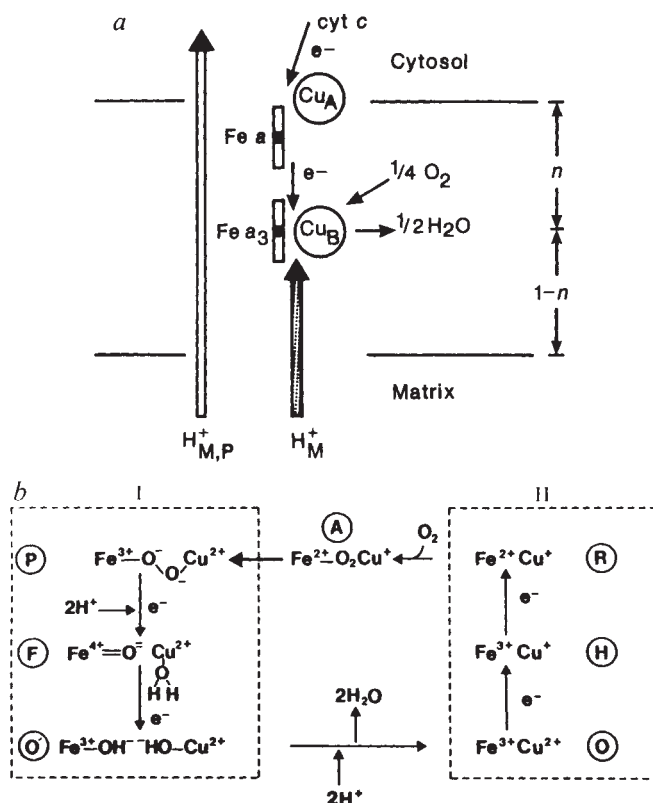


FIG. 1 a, Electrogenic reactions of cytochrome oxidase. Each electron transferred from cytochrome *c*, through haem *a* (Fe *a*) and Cu_A, to the bimetallic haem *a*₃ (Fe *a*₃)-Cu_B centre, and reduction of O₂ to water, is accompanied by the uptake of 1 H⁺ from the matrix phase for the formation of water (shaded arrow), and the pumping of 1 H⁺ across the membrane (white arrow). The fraction of the total transmembrane dielectric traversed by electron transfer from cytochrome *c* to the bimetallic centre is denoted by *n*. Proton uptake to the centre traverses the fraction (1-*n*). b, Proposed catalytic scheme of O₂ reduction by the bimetallic centre. Fe and Cu denote the iron of haem *a*₃ and Cu_B, respectively. The main intermediates previously observed spectroscopically are shown. O and O' are not distinguishable by optical spectroscopy, but by the pH-dependence of the O→F transition. Uptake of H⁺ corresponds to H_M⁺ in a. Proton-pumping is not shown. Dashed boxes labelled I and II outline the parts of the cycle that are here proposed to be coupled and not coupled to proton-pumping, respectively. Further details are described in refs 5–7, 10.

TABLE 1 Calculation of the H⁺/e⁻ ratio of proton-pumping

Reaction step	H ⁺ uptake in reaction H _M ⁺	Charge translocated in reaction q ⁺	Observed total H ⁺ translocated H _{obs} ⁺	H ⁺ pumped H _{M,P} ⁺	Charge pumped q _P ⁺
F→O (pH 7.2)	1	1	2.8–3.0	1.8–2.0	1.8–2.0
P→F (pH 8.3)	2	1.5	3.6–4.0	1.6–2.0	2.1–2.5

H_M⁺ is the number of H⁺ taken up from the matrix side of the membrane at the indicated pH, excluding proton-pumping, as determined previously⁷ (see Fig. 1a, b). q⁺ is the number of electrical charge equivalents translocated, excluding proton-pumping, linked to the corresponding reaction, and assuming *n*=0.5 (Fig. 1a). H_{obs}⁺ denotes the observed total number of H⁺ translocated (Fig. 2), assuming a H⁺/ATP ratio of 4. H_{M,P}⁺ is the number of H⁺ taken up from the matrix side due to proton-pumping alone (Fig. 1a), obtained as H_{obs}⁺-H_M⁺. q_P⁺ is the number of electrical charge equivalents translocated due to proton-pumping alone, obtained as H_{obs}⁺-q⁺. Due to inequivalency of q⁺ and H_M⁺, and the dominance of the $\Delta\psi$ term over the ΔpH term of the proton-motive force, H_{M,P}⁺ underestimates, and q_P⁺ overestimates the H⁺ pump stoichiometry for the P→F transition.

ate O' dominates over O (ref. 7) (Fig. 1b) so that the only linked electrogenic event expected apart from proton-pumping is electron transfer to cytochrome *c*. The 27-mV dependence observed indicates that the oxidation of O' to F by cytochrome *c* is coupled to the translocation of ~ 2.2 (60 mV/27 mV) charge equivalents. Subtraction of the fraction due to electron transfer (see legend to Table 1 and Fig. 1a) yields 1.7 charges translocated by proton-pumping, in accordance with the data in Table 1.

This work shows that the four electron transfers from cytochrome *c* to the bimetallic centre (Fig. 1) are non-equivalent with respect to coupling to proton-pumping. The steps reducing the 'peroxy' and 'oxyferryl' intermediates are probably each coupled to translocation of 2 H⁺, whereas the two other electron transfers of the catalytic cycle (Fig. 1b, box II) are not coupled to proton-pumping.

It is generally assumed that each electron is transferred equivalently from cytochrome *c* first to the Cu_A and haem *a* redox centres, and then to the bimetallic centre. With respect to the latter, however, the four electron transfers are non-equivalent (Fig. 1b), suggesting that neither Cu_A nor haem *a* can *a priori* have primary mechanistic roles in proton-pumping. Instead, it seems that the bimetallic centre is itself critically involved in the mechanism. There are, however, two alternative possibilities by which either Cu_A or haem *a* could play a part in proton-pumping without excluding the bimetallic centre. First, it is conceivable that either Cu_A or haem *a* might specifically carry electrons from cytochrome *c* to P and F, but not to O or H, although there is no experimental support for this at present. Second, there might be some co-operativity between Cu_A or haem *a* with the bimetallic centre. The latter has been demonstrated, whereas interactions with Cu_A are small or non-existent¹¹⁻¹⁴. Haem *a* could thus have a specific configuration when oxygen is bound to the bimetallic centre (states P and F), and could therefore be mechanistically involved in proton-pumping^{15,16}.

The observed 'mechanistic' 2 H⁺/e⁻ ratio in itself obviously constrains possible mechanisms of proton-pumping. Interestingly, proton-pumping by bacteriorhodopsin has the same stoichiometry per photocycle. Here the mechanism includes

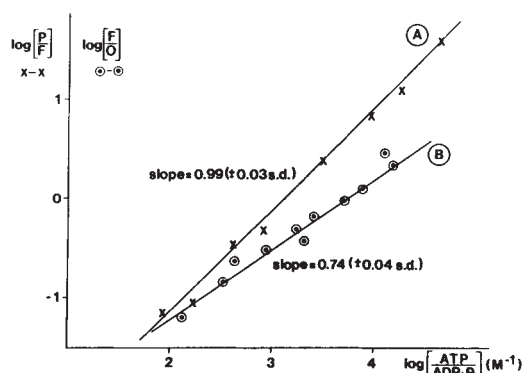


FIG. 2 Dependence of the P/F (A) and F/O (B) equilibria on phosphorylation potential. Rat liver mitochondria (0.77 μ M cytochrome *aa*₃) were suspended in 0.2 M sucrose-20 mM KCl-20 mM HEPES-Tris buffer in the presence of 3 μ M rotenone, 0.25 μ g ml⁻¹ antimycin, 5 μ M myxothiazol, 0.75 mM EGTA and 0.96 mM potassium phosphate. Reaction temperature, 25°. In A, 1.5 mM potassium ferricyanide was also present (pH 8.3). In B, 1.5 mM each of potassium ferricyanide and ferrocyanide were present (pH 7.2). Varying amounts of ADP and ATP were added in different incubations, and the corresponding concentrations of P, F, and O determined by deconvolution⁷ of spectroscopic data at 583 and 607 nm, with 630 nm as reference. The straight lines are derived from a linear regression analysis of the data. The slopes (varying between 0.9 and 1 for A and between 0.70 and 0.75 for B in different experiments) indicate the number of ATP molecules hydrolysed per molecular event.

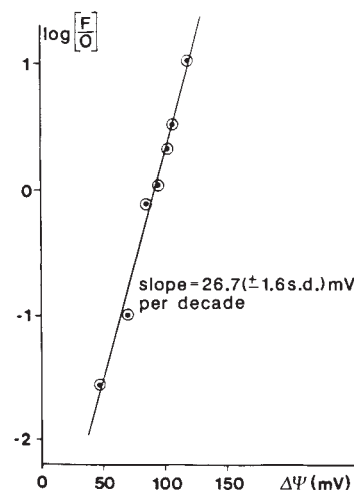


FIG. 3 Dependence of the F/O equilibrium on membrane potential. Rat liver mitochondria were suspended in 0.25 M sucrose-20 mM HEPES-Tris buffer at pH 8.3, in the presence of rotenone, antimycin, myxothiazol and EGTA (see Fig. 2), and 1.5 mM sodium ferricyanide. After 15 s 0.4 μ g ml⁻¹ of valinomycin was added, and ΔA recorded as described for Fig. 2. KCl was added at different concentrations to vary $\Delta\psi$. Without added KCl the extramitochondrial K⁺ concentration was 1 mM after adding valinomycin (as measured potentiometrically). Much of this K⁺ stems from the intramitochondrial space and part stems from the buffer used to isolate the mitochondria. The amounts of F and O were determined at each potential, as described for Fig. 2; in these conditions only negligible amounts of P were formed. $\Delta\psi$ was estimated from the Nernst equation, assuming 120 mM intramitochondrial K⁺. Linear regression analysis gave the line of best fit shown.

active participation of both the chromophore and acidic amino-acid residues in the apoprotein¹⁷. Directly coupled mechanisms, which typically involve the redox centres, the substrates and the products¹⁸⁻²¹, are more strongly circumscribed by the H⁺/e⁻ ratio of 2 than are indirect mechanisms with essential contributions from the protein structure. This is not only because it is easier, at present, to explicitly describe directly coupled mechanisms chemically, but also because direct coupling of two-proton transfer to one-electron transfer is less common in biological chemistry. Yet, such coupling does exist, as exemplified by the superoxide/hydrogen peroxide redox couple at physiological pH. □

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- Wikström, M. *Nature* **266**, 271-273 (1977).
- Chance, B., Saronio, C. & Leigh, J. S. Jr. *J. biol. Chem.* **250**, 9,226-9,237 (1975).
- Clore, G. M., Andreasson, L.-E., Karlsson, B., Aasa, R. & Malmström, B. G. *Biochem. J.* **185**, 139-154, 155-167 (1980).
- Blair, D. F., Witt, S. N. & Chan, S. I. *J. Am. chem. Soc.* **107**, 7,389-7,399 (1985).
- Wikström, M. *Proc. natn. Acad. Sci. USA* **78**, 4,051-4,054 (1981).
- Wikström, M. *Chem. Scr.* **27B**, 53-58 (1987).
- Wikström, M. *Chem. Scr.* **28A**, 71-74 (1988).
- Chamalaun, R. A. F. M. & Tager, J. M. *Biochim. biophys. Acta* **180**, 204-206 (1969).
- Kagawa, Y. in *Bioenergetics, New Comprehensive Biochemistry* Vol. 9 (ed. Ernster, L.) 149-186 (Elsevier, 1984).
- Wikström, M. *FEBS Lett.* **231**, 247-252 (1988).
- Wikström, M., Harmon, H. J., Ingledew, W. J. & Chance, B. *FEBS Lett.* **65**, 259-277 (1976).
- Goodman, G. *J. biol. Chem.* **259**, 15,094-15,099 (1984).
- Blair, D. F. *J. biol. Chem.* **261**, 11,524-11,537 (1986).
- Wikström, M. *Ann. N. Y. Acad. Sci.* (in the press).
- Callahan, P. M. & Babcock, G. T. *Biochemistry* **22**, 452-461 (1983).
- Wikström, M. & Casey, R. P. *J. inorg. Biochem.* **23**, 327-334 (1985).
- Engelhard, M., Gerwert, K., Hess, B., Kreutz, W. & Siebert, F. *Biochemistry* **24**, 400-407 (1985).
- Mitchell, P. et al. *FEBS Lett.* **188**, 1-7 (1985).
- Mitchell, P. *Glynn Biol. Res. Rep.* **3**, 1-6 (1987).
- Krab, K. & Wikström, M. *Biochim. biophys. Acta* **895**, 25-39 (1988).
- Mitchell, P. *FEBS Lett.* **222**, 235-245 (1987).

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